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(54) Title: PATCHED GENES AND THEIR USES			
(57) Abstract			
<p>Methods for isolating patched genes, particularly mammalian patched genes, including the mouse and human <i>patched</i> genes, as well as invertebrate patched genes and sequences, are provided. Decreased expression of <i>patched</i> is associated with the occurrence of human cancers, particularly basal cell carcinomas of the skin. The cancers may be familial, having as a component of risk an inherited genetic predisposition, or may be sporadic. The <i>patched</i> and <i>hedgehog</i> genes are useful in creating transgenic animal models for these human cancers. The <i>patched</i> nucleic acid compositions find use in identifying homologous or related proteins and the DNA sequences encoding such proteins; in producing compositions that modulate the expression or function of the protein; and in studying associated 15 physiological pathways. In addition, modulation of the gene activity <i>in vivo</i> is used for prophylactic and therapeutic purposes, such as treatment of cancer, identification of cell type based on expression, and the like. The DNA is further used as a diagnostic for a genetic predisposition to cancer, and to identify specific cancers having mutations in this gene.</p>			



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PATCHED GENES AND THEIR USES

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INTRODUCTION

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Technical Field

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The field of this invention is segment polarity genes and their uses.

Background

Segment polarity genes were originally discovered as mutations in flies that change the
15 pattern of body segment structures. Mutations in these genes cause animals to develop changed patterns on the surfaces of body segments; the changes affecting the pattern along the head to tail axis. Among the genes in this class are *hedgehog*, which encodes a secreted protein (HH), and *patched*, which encodes a protein structurally similar to transporter proteins, having twelve transmembrane domains (*ptc*), with two conserved glycosylation signals.

20 The *hedgehog* gene of flies has at least three vertebrate relatives- *Sonic hedgehog* (*Shh*); *Indian hedgehog* (*Ihh*), and *Desert hedgehog* (*Dhh*). *Shh* is expressed in a group of cells, at the posterior of each developing limb bud, that have an important role in signaling polarity to the developing limb. The *Shh* protein product, SHH, is a critical trigger of posterior limb development, and is also involved in polarizing the neural tube and somites along the dorsal
25 ventral axis. Based on genetic experiments in flies, *patched* and *hedgehog* have antagonistic effects in development. The *patched* gene product, *ptc*, is widely expressed in fetal and adult tissues, and plays an important role in regulation of development. *Ptc* downregulates

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5 transcription of itself, members of the transforming growth factor β and *Wnt* gene families, and possibly other genes. Among other activities, HH upregulates expression of *patched* and other genes that are negatively regulated by *patched*.

It is of interest that many genes involved in the regulation of growth and control of cellular signaling are also involved in oncogenesis. Such genes may be oncogenes, which are
10 typically upregulated in tumor cells, or tumor suppressor genes, which are down-regulated or absent in tumor cells. Malignancies may arise when a tumor suppressor is lost and/or an oncogene is inappropriately activated. Familial predisposition to cancer may occur when there is a mutation, such as loss of an allele encoding a suppressor gene, present in the germline DNA of an individual.

15 The most common form of cancer in the United States is basal cell carcinoma of the skin. While sporadic cases are very common, there are also familial syndromes, such as the basal cell nevus syndrome (BCNS). The familial syndrome has many features indicative of abnormal embryonic development, indicating that the mutated gene also plays an important role in development of the embryo. A loss of heterozygosity of chromosome 9q alleles in both familial
20 and sporadic carcinomas suggests that a tumor suppressor gene is present in this region. The high incidence of skin cancer makes the identification of this putative tumor suppressor gene of great interest for diagnosis, therapy, and drug screening.

Relevant Literature

Descriptions of *patched*, by itself or its role with *hedgehog* may be found in Hooper and
25 Scott (1989) *Cell* 59:751-765; and Nakano *et al.* (1989) *Nature* 341:508-513. Both of these references also describe the sequence for *Drosophila patched*. Discussions of the role of *hedgehog* include Riddle *et al.* (1993) *Cell* 75:1401-1416, Echelard *et al.* (1993) *Cell* 75:1417-1430, Krauss *et al.* (1993) *Cell* 75:1431-1444 (1993); Tabata and Kornberg (1994) 76:89-102;

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- 5 Heemskerk and DiNardo (1994) Cell 76:449-460; and Roelink *et al.* (1994) Cell 76:-761-775.

Mapping of deleted regions on chromosome 9 in skin cancers is described in Habuchi *et al.* (1995) Oncogene 11: 1 671-1674, Quinn *et al.* (1 994) Genes Chromosome Cancer 11:222-225; Quinn *et al.* (1994) J. Invest. Dermatol. 102:300-303; and Wicking *et al.* (1994) Genomics 22:505-51 1.

- 10 Gorlin (1987) Medicine 66:98-113 reviews nevoid basal cell carcinoma syndrome. The syndrome shows autosomal dominant inheritance with probably complete penetrance. About 60% of the cases represent new mutations. Developmental abnormalities found with this syndrome include rib and craniofacial abnormalities, polydactyly, syndactyly and spina bifida. Tumors found with the syndrome include basal cell carcinomas, fibromas of the ovaries and
- 15 heart, cysts of the skin, jaws and mesentery, meningiomas and medulloblastomas.

SUMMARY OF THE INVENTION

Isolated nucleotide compositions and sequences are provided for *patched (ptc)* genes, including mammalian, e.g. human and mouse, and invertebrate homologs. Decreased

20 expression of *ptc* is associated with the occurrence of human cancers, particularly basal cell carcinomas and other tumors of epithelial tissues such as the skin. The cancers may be familial, having as a component of risk a germline mutation in the gene, or may be sporadic. *Ptc*, and its antagonist *hedgehog*, are useful in creating transgenic animal models for these human cancers. The *ptc* nucleic acid compositions find use in identifying homologous or

25 related genes; in producing compositions that modulate the expression or function of its encoded protein, *ptc*; for gene therapy; mapping functional regions of the protein- and in studying associated physiological pathways. In addition, modulation of the gene activity *in vivo* is used

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5 for prophylactic and therapeutic purposes, such as treatment of cancer, identification of cell type based on expression, and the like. *Ptc*, anti-*ptc* antibodies and *ptc* nucleic acid sequences are useful as diagnostics for a genetic predisposition to cancer or developmental abnormality syndromes, and to identify specific cancers having mutations in this gene.

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BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a graph having a restriction map of about 10 kbp of the 5' region upstream from the initiation codon of *Drosophila patched* gene and bar graphs of constructs of truncated portions of the 5' region joined to fl-galactosidase, where the constructs are introduced into fly cell lines for the production of embryos. The expression of fl-gal in the embryos is indicated
15 in the right-hand table during early and late development of the embryo. The greater the number of +'s, the more intense the staining.

Fig. 2 shows a summary of mutations found in the human *patched* gene locus that are associated with basal cell nevus syndrome. Mutation (1) is found in sporadic basal cell carcinoma, and is a C to T transition in exon 3 at nucleotide 523 of the coding sequence,
20 changing Leu 175 to Phe in the first extracellular loop. Mutations 2-4 are found in hereditary basal carcinoma nevus syndrome. (2) is an insertion of 9 bp at nucleotide 2445, resulting in the insertion of an additional 3 amino acids after amino acid 815. (3) is a deletion of 11 bp, which removes nt 2442-2452 from the coding sequence. The resulting frameshift truncates the open reading frame after amino acid 813, just after the seventh transmembrane domain. (4) is a G to
25 C alteration that changes two conserved nucleotides of the 3' splice site adjacent to exon 10, creating a non-functional splice site that truncates the protein after amino acid 449, in the second transmembrane region.

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5 DATABASE REFERENCES FOR NUCLEOTIDE AND AMINO ACID SEQUENCES

The sequence for the *D. melanogaster patched* gene has the Genbank accession number M28418. The sequence for the mouse *patched* gene has the Genbank accession number U30589-V46155. The sequence for the human *patched* gene has the Genbank accession number U59464.

10

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

Mammalian and invertebrate *patched (ptc)* gene compositions and methods for their isolation are provided. Of particular interest are the human and mouse homologs. Certain human cancers, e.g. basal cell carcinoma, transitional cell carcinoma of the bladder, 15 meningiomas, medulloblastomas, etc., show decreased *ptc* activity, resulting from oncogenic mutations at the *ptc* locus. Many such cancers are sporadic, where the tumor cells have a somatic mutation in *ptc*. The basal cell nevus syndrome (BCNS), an inherited disorder, is associated with germline mutations in *ptc*. Such germline mutations may also be associated with other human cancers, including carcinomas, adenocarcinomas, sarcomas and the like. 20 Decreased *ptc* activity is also associated with inherited developmental abnormalities, e.g. rib and craniofacial abnormalities, polydactyly, syndactyly and spina bifida.

The *ptc* genes and fragments thereof, encoded protein, and anti-*ptc* antibodies are useful in the identification of individuals predisposed to development of such cancers and developmental abnormalities, and in characterizing the phenotype of sporadic tumors that are 25 associated with this gene, e.g., for diagnostic and/or prognostic benefit. The characterization is useful for prenatal screening, and in determining further treatment of the patient. Tumors may be typed or staged as to the *ptc* status, e.g. by detection of mutated sequences, antibody detection of abnormal protein products, and functional assays for altered *ptc* activity. The

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5 encoded *ptc* protein is useful in drug screening for compositions that mimic *ptc* activity or expression, including altered forms of *ptc* protein, particularly with respect to *ptc* function as a tumor suppressor in oncogenesis.

The human and mouse *ptc* gene sequences and isolated nucleic acid compositions are provided. In identifying the mouse and human *patched* genes, cross-hybridization of DNA and
10 amplification primers were employed to move through the evolutionary tree from the known *Drosophila ptc* sequence, identifying a number of invertebrate homologs. The human *patched* gene has been mapped to human chromosome band 9q22.3, and lies between the polymorphic markers D9S196 and D9S287 (a detailed map of human genome markers may be found in Dib *et al.* (1996) Nature 280-152-1 <http://www.genethon.fr>).

15 DNA from a patient having a tumor or developmental abnormality, which may be associated with *ptc*, is analyzed for the presence of a predisposing mutation in the *ptc* gene. The presence of a mutated *ptc* sequence that affects the activity or expression of the gene product, *ptc*, confers an increased susceptibility to one or more of these conditions. Individuals are screened by analyzing their DNA for the presence of a predisposing oncogenic or
20 developmental mutation, as compared to a normal sequence. A "normal" sequence of *patched* is provided in SEQ ID NO-18 (human). Specific mutations of interest include any mutation that leads to oncogenesis or developmental abnormalities, including insertions, substitutions and deletions in the coding region sequence, introns that affect splicing, promoter or enhancer that affect the activity and expression of the protein.

25 Screening for tumors or developmental abnormalities may also be based on the functional or antigenic characteristics of the protein. Immunoassays designed to detect the normal or abnormal *ptc* protein may be used in screening. Where many diverse mutations lead to a particular disease phenotype, functional protein assays have proven to be effective screening

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5 tools. Such assays may be based on detecting changes in the transcriptional regulation mediated by *ptc*, or may directly detect *ptc* transporter activity, or may involve antibody localization of *patched* in cells.

Inheritance of BCNS is autosomal dominant, although many cases are the result of new mutations. Diagnosis of BCNS is performed by protein, DNA sequence or hybridization
10 analysis of any convenient sample from a patient, e.g. biopsy material, blood sample, scrapings from cheek, etc. A typical patient genotype will have a predisposing mutation on one chromosome. In tumors and at least sometimes developmentally affected tissues, loss of heterozygosity at the *ptc* locus leads to aberrant cell and tissue behavior. When the normal copy of *ptc* is lost, leaving only the reduced function mutant copy, abnormal cell growth and
15 reduced cell layer adhesion is the result. Examples of specific *ptc* mutations in BCNS patients are a 9 bp insertion at nt 2445 of the coding sequence- and an 11 bp deletion of nt 2441 to 2452 of the coding sequence. These result in insertions or deletions in the region of the seventh transmembrane domain.

Prenatal diagnosis of BCNS may be performed, particularly where there is a family
20 history of the disease, e.g. an affected parent or sibling. It is desirable, although not required, in such cases to determine the specific predisposing mutation present in affected family members. A sample of fetal DNA, such as an amniocentesis sample, fetal nucleated or white blood cells isolated from maternal blood, chorionic villus sample, etc. is analyzed for the presence of the predisposing mutation. Alternatively, a protein based assay, e.g. functional
25 assay or immunoassay, is performed on fetal cells known to express *ptc*.

Sporadic tumors associated with loss of *ptc* function include a number of carcinomas and other transformed cells known to have deletions in the region of chromosome 9q22, e.g. basal cell carcinomas, transitional bladder cell carcinoma, meningiomas, medullomas, fibromas of the

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5 heart and ovary, and carcinomas of the lung, ovary, kidney and esophagus. Characterization of sporadic tumors will generally require analysis of tumor cell DNA, conveniently with a biopsy sample. A wide range of mutations are found in sporadic cases, up to and including deletion of the entire long arm of chromosome 9. Oncogenic mutations may delete one or more exons, e.g. 8 and 9, may affect the amino acid sequence such as of the extracellular loops or
10 transmembrane domains, may cause truncation of the protein by introducing a frameshift or stop codon, etc. Specific examples of oncogenic mutations include a C to T transition at nt 523-1 and deletions encompassing exon 9. C to T transitions are characteristic of ultraviolet mutagenesis, as expected with cases of skin cancer.

Biochemical studies may be performed to determine whether a candidate sequence
15 variation in the *ptc* coding region or control regions is oncogenic. For example, a change in the promoter or enhancer sequence that downregulates expression of *patched* may result in predisposition to cancer. Expression levels of a candidate variant allele are compared to expression levels of the normal allele by various methods known in the art. Methods for determining promoter or enhancer strength include quantitation of the expressed natural protein;
20 insertion of the variant control element into a vector with a reporter gene such as R-galactosidase, chloramphenical acetyltransferase, etc. that provides for convenient quantitation and the like. The activity of the encoded *ptc* protein may be determined by comparison with the wild-type protein, e.g. by detection of transcriptional down-regulation of TGFP, *Wnt* family genes, *ptc* itself, or reporter gene fusions involving these target genes.

25 The human *patched* gene (SEQ ID NO:18) has a 4.5 kb open reading frame encoding a protein of 1447 amino acids. Including coding and noncoding sequences, it is about 89% identical at the nucleotide level to the mouse *patched* gene (SEQ ID NO:09). The mouse *patched* gene (SEQ ID NO:09) encodes a protein (SEQ ID NO:10) that has about 38% identical

5 amino acids to *Drosophila ptc* (SEQ ID NO:6), over about 1,200 amino acids. The butterfly homolog (SEQ ID NO:4) is 1,300 amino acids long and overall has a 50% amino acid identity to fly *ptc* (SEQ ID NO:6). A 267 bp exon from the beetle patched gene encodes an 89 amino acid protein fragment, which was found to be 44% and 51% identical to the corresponding regions of fly and butterfly *ptc* respectively.

10 The DNA sequence encoding *ptc* may be cDNA or genomic DNA or a fragment thereof. The term "*patched gene*" shall be intended to mean the open reading frame encoding specific *ptc* polypeptides, as well as adjacent 5' and 3' non-coding nucleotide sequences involved in the regulation of expression, up to about 1 kb beyond the coding region, in either direction. The gene may be introduced into an appropriate vector for extrachromosomal maintenance or for
15 integration into the host.

The term "cDNA" as used herein is intended to include all nucleic acids that share the arrangement of sequence elements found in native mature mRNA species, where sequence elements are exons, 3' and 5' non-coding regions. Normally MRNA species have contiguous exons, with the intervening introns deleted, to create a continuous open reading frame encoding
20 *ptc*.

The genomic *ptc* sequence has non-contiguous open reading frames, where introns interrupt the coding regions. A genomic sequence of interest comprises the nucleic acid present between the initiation codon and the stop codon, as defined in the listed sequences, including all of the introns that are normally present in a native chromosome. It may further include the
25 3' and 5' untranslated regions found in the mature MRNA. It may further include specific transcriptional and translational regulatory sequences, such as promoters, enhancers, etc., including about 1 kb of flanking genomic DNA at either the 5' or 3' end of the coding region. The genomic DNA may be isolated as a fragment of 50 kbp or smaller, and substantially free

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5 of flanking chromosomal sequence.

The nucleic acid compositions of the subject invention encode all or a part of the subject polypeptides. Fragments may be obtained of the DNA sequence by chemically synthesizing oligonucleotides in accordance with conventional methods, by restriction enzyme digestion, by PCR amplification, etc. For the most part, DNA fragments will be of at least 15 nt, usually at
10 least 18 nt, more usually at least about 50 nt. Such small DNA fragments are useful as primers for PCR, hybridization screening, etc. Larger DNA fragments, i.e. greater than 100 nt are useful for production of the encoded polypeptide. For use in amplification reactions, such as PCR, a pair of primers will be used. The exact composition of the primer sequences is not critical to the invention, but for most applications the primers will hybridize to the subject
15 sequence under stringent conditions, as known in the art. It is preferable to chose a pair of primers that will generate an amplification product of at least about 50 nt, preferably at least about 100 nt. Algorithms for the selection of primer sequences are generally known, and are available in commercial software packages. Amplification primers hybridize to complementary strands of DNA, and will prime towards each other.

20 The *ptc* genes are isolated and obtained in substantial purity, generally as other than an intact mammalian chromosome. Usually, the DNA will be obtained substantially free of other nucleic acid sequences that do not include a *ptc* sequence or fragment thereof, generally being at least about 50%, usually at least about 90% pure and are typically "recombinant", i.e. flanked by one or more nucleotides with which it is not normally associated on a naturally occurring
25 chromosome.

The DNA sequences are used in a variety of ways. They may be used as probes for identifying other patched genes. Mammalian homologs have substantial sequence similarity to the subject sequences, i.e. at least 75%, usually at least 90%, more usually at least 95%

5 sequence identity with the nucleotide sequence of the subject DNA sequence. Sequence similarity is calculated based on a reference sequence, which may be a subset of a larger sequence, such as a conserved motif, coding region, flanking region, etc. A reference sequence will usually be at least about 18 nt long, more usually at least about 30 nt long, and may extend to the complete sequence that is being compared. Algorithms for sequence analysis are known
10 in the art, such as BLAST, described in Altschul *et al.* (1990) *J Mol Biol* 215: 403-10.

Nucleic acids having sequence similarity are detected by hybridization under low stringency conditions, for example, at 50°C and 10XSSC (0.9 M saline/0.09 M sodium citrate) and remain bound when subjected to washing at 55°C in 1XSSC. By using probes, particularly labeled probes of DNA sequences, one can isolate homologous or related genes. The source of
15 homologous genes may be any mammalian species, e.g. primate species, particularly human-murines, such as rats and mice, canines, felines, bovines, ovines, equines, etc.

The DNA may also be used to identify expression of the gene in a biological specimen. The manner in which one probes cells for the presence of particular nucleotide sequences, as genomic DNA or RNA, is well-established in the literature and does not require elaboration
20 here. Conveniently, a biological specimen is used as a source of mRNA. The mRNA may be amplified by RT-PCR, using reverse transcriptase to form a complementary DNA strand, followed by polymerase chain reaction amplification using primers specific for the subject DNA sequences. Alternatively, the mRNA sample is separated by gel electrophoresis, transferred to a suitable support, e.g., nitrocellulose and then probed with a fragment of the subject DNA as
25 a probe. Other techniques may also find use. Detection of mRNA having the subject sequence is indicative of *patched* gene expression in the sample.

The subject nucleic acid sequences may be modified for a number of purposes, particularly where they will be used intracellularly, for example, by being joined to a nucleic acid

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5 cleaving agent, e.g. a chelated metal ion, such as iron or chromium for cleavage of the gene; as an antisense sequence-, or the like. Modifications may include replacing oxygen of the phosphate esters with sulfur or nitrogen, replacing the phosphate with phosphoramidate, etc.

A number of methods are available for analyzing genomic DNA sequences. Where large amounts of DNA are available, the genomic DNA is used directly. Alternatively, the region of
10 interest is cloned into a suitable vector and grown in sufficient quantity for analysis, or amplified by conventional techniques, such as the polymerase chain reaction (PCR). The use of the polymerase chain reaction is described in Saiki, *et al.* (1985) *Science* 239:487, and a review of current techniques may be found in Sambrook, *et al.* Molecular Cloning: A Laboratory Manual, CSH Press 1989, pp.14.2-14.33.

15 A detectable label may be included in the amplification reaction. Suitable labels include fluorochromes, e.g. fluorescein isothiocyanate (FITC), rhodamine, Texas Red, phycoerythrin, allophycocyanin, 6-carboxyfluorescein (6-FAM), 2',7'-dimethoxy-4',5'-dichloro-6-carboxyfluorescein (JOE), 6-carboxy-Xrhodamine (ROX), 6-carboxy-2',4',7',4',7-hexachlorofluorescein (HEX), 5-carboxyfluorescein (5-FAM) or N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA), radioactive labels, e.g. ^{32}P , ^{35}S , ^3H ; etc. The label may be a two
20 stage system, where the amplified DNA is conjugated to biotin, haptens, etc. having a high affinity binding partner, e.g. avidin, specific antibodies, etc., where the binding partner is conjugated to a detectable label. The label may be conjugated to one or both of the primers. Alternatively, the pool of nucleotides used in the amplification is labeled, so as to incorporate
25 the label into the amplification product.

The amplified or cloned fragment may be sequenced by dideoxy or other methods, and the sequence of bases compared to the normal *ptc* sequence. Hybridization with the variant sequence may also be used to determine its presence, by Southern blots, dot blots, etc. Single

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5 strand conformational polymorphism (SSCP) analysis, denaturing gradient gel electrophoresis (DGGE), and heteroduplex analysis in gel matrices are used to detect conformational changes created by DNA sequence variation as alterations in electrophoretic mobility. The hybridization pattern of a control and variant sequence to an array of oligonucleotide probes immobilized on a solid support, as described in WO 95/11995, may also be used as a means of detecting the
10 presence of variant sequences. Alternatively, where a predisposing mutation creates or destroys a recognition site for a restriction endonuclease, the fragment is digested with that endonuclease, and the products size fractionated to determine whether the fragment was digested. Fractionation is performed by gel electrophoresis, particularly acrylamide or agarose gels.

The subject nucleic acids can be used to generate transgenic animals or site specific gene
15 modifications in cell lines. Transgenic animals may be made through homologous recombination, where the normal patched locus is altered. Alternatively, a nucleic acid construct is randomly integrated into the genome. Vectors for stable integration include plasmids, retroviruses and other animal viruses, YACS, and the like.

The modified cells or animals are useful in the study of *patched* function and regulation.
20 For example, a series of small deletions and/or substitutions may be made in the *patched* gene to determine the role of different exons in oncogenesis, signal transduction, etc. Of particular interest are transgenic animal models for carcinomas of the skin, where expression of *ptc* is specifically reduced or absent in skin cells. An alternative approach to transgenic models for this disease are those where one of the mammalian *hedgehog* genes, e.g. *Shh*, *lhh*, *Dhh*, are
25 upregulated in skin cells, or in other cell types. For models of skin abnormalities, one may use a skin-specific promoter to drive expression of the transgene, or other inducible promoter that can be regulated in the animal model. Such promoters include keratin gene promoters. Specific constructs of interest include anti-sense *ptc*, which will block *ptc* expression, expression of

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5 dominant negative *ptc* mutations, and over-expression of HH genes. A detectable marker, such as *lacZ* may be introduced into the *patched* locus, where upregulation of patched expression will result in an easily detected change in phenotype.

One may also provide for expression of the *patched* gene or variants thereof in cells or tissues where it is not normally expressed or at abnormal times of development. Thus, mouse
10 models of spina bifida or abnormal motor neuron differentiation in the developing spinal cord are made available. In addition, by providing expression of *ptc* protein in cells in which it is otherwise not normally produced, one can induce changes in cell behavior, e.g. through *ptc* mediated transcription modulation.

DNA constructs for homologous recombination will comprise at least a portion of the
15 *patched* or *hedgehog* gene with the desired genetic modification, and will include regions of homology to the target locus. DNA constructs for random integration need not include regions of homology to mediate recombination. Conveniently, markers for positive and negative selection are included. Methods for generating cells having targeted gene modifications through homologous recombination are known in the art. For various techniques for transfecting
20 mammalian cells, see Keown *et al.* (1990) *Methods in Enzymology* 185:527-537.

For embryonic stem (ES) cells, an ES cell line may be employed, or ES cells may be obtained freshly from a host, e.g. mouse, rat, guinea pig, etc. Such cells are grown on an appropriate fibroblast-feeder layer or grown in the presence of leukemia inhibiting factor (LIF). When ES cells have been transformed, they may be used to produce transgenic animals. After
25 transformation, the cells are plated onto a feeder layer in an appropriate medium. Cells containing the construct may be detected by employing a selective medium. After sufficient time for colonies to grow, they are picked and analyzed for the occurrence of homologous recombination or integration of the construct. Those colonies that are positive may then be used

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5 for embryo manipulation and blastocyst injection. Blastocysts are obtained from 4 to 6 week old superovulated females. The ES cells are trypsinized, and the modified cells are injected into the blastocoel of the blastocyst. After injection, the blastocysts are returned to each uterine horn of pseudopregnant females. Females are then allowed to go to term and the resulting litters screened for mutant cells having the construct. By providing for a different phenotype of the
10 blastocyst and the ES cells, chimeric progeny can be readily detected.

The chimeric animals are screened for the presence of the modified gene and males and females having the modification are mated to produce homozygous progeny. If the gene alterations cause lethality at some point in development, tissues or organs can be maintained as allogeneic or congenic grafts or transplants, or in *in vitro* culture. The transgenic animals may
15 be any non-human mammal, such as laboratory animals, domestic animals, etc. The transgenic animals may be used in functional studies, drug screening, etc., e.g. to determine the effect of a candidate drug on basal cell carcinomas.

The subject gene may be employed for producing all or portions of the patched protein. For expression, an expression cassette may be employed, providing for a transcriptional and
20 translational initiation region, which may be inducible or constitutive, the coding region under the transcriptional control of the transcriptional initiation region, and a transcriptional and translational termination region. Various transcriptional initiation regions may be employed which are functional in the expression host.

Specific *ptc* peptides of interest include the extracellular domains, particularly in the
25 human mature protein, aa 120 to 437, and aa 770 to 1027. These peptides may be used as immunogens to raise antibodies that recognize the protein in an intact cell membrane. The cytoplasmic domains, as shown in Figure 2, (the amino terminus and carboxy terminus) are of interest in binding assays to detect ligands involved in signaling mediated by *ptc*.

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5 The peptide may be expressed in prokaryotes or eukaryotes in accordance with conventional ways, depending upon the purpose for expression. For large scale production of the protein, a unicellular organism or cells of a higher organism, e.g. eukaryotes such as vertebrates, particularly mammals, may be used as the expression host, such as *E. coli*, *B. subtilis*, *S. cerevisiae*, and the like. In many situations, it may be desirable to express the *patched* gene in a mammalian host, whereby the *patched* gene will be glycosylated, and transported to
10 the cellular membrane for various studies.

With the availability of the protein in large amounts by employing an expression host, the protein may be isolated and purified in accordance with conventional ways. A lysate may be prepared of the expression host and the lysate purified using HPLC, exclusion chromatography,
15 gel electrophoresis, affinity chromatography, or other purification technique. The purified protein will generally be at least about 80% pure, preferably at least about 90% pure, and may be up to and including 100% pure. By pure is intended free of other proteins, as well as cellular debris.

The polypeptide is used for the production of antibodies, where short fragments provide
20 for antibodies specific for the particular polypeptide, whereas larger fragments or the entire gene allow for the production of antibodies over the surface of the polypeptide or protein. Antibodies may be raised to the normal or mutated forms of *ptc*. The extracellular domains of the protein are of interest as epitopes, particular antibodies that recognize common changes found in abnormal, oncogenic *ptc*, which compromise the protein activity. Antibodies may be raised to
25 isolated peptides corresponding to these domains, or to the native protein, e.g. by immunization with cells expressing *ptc*, immunization with liposomes having *ptc* inserted in the membrane, etc. Antibodies that recognize the extracellular domains of *ptc* are useful in diagnosis, typing and staging of human carcinomas.

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5 Antibodies are prepared in accordance with conventional ways, where the expressed polypeptide or protein may be used as an immunogen, by itself or conjugated to known immunogenic carriers, e.g. KLH, pre-S HBsAg, other viral or eukaryotic proteins, or the like. Various adjuvants may be employed, with a series of injections, as appropriate. For monoclonal antibodies, after one or more booster injections, the spleen may be isolated, the splenocytes
10 immortalized, and then screened for high affinity antibody binding. The immortalized cells, e.g. hybridomas, producing the desired antibodies may then be expanded. For further description, see *Monoclonal Antibodies- A Laboratory Manual*, Harlow and Lane eds., Cold Spring Harbor Laboratories, Cold Spring Harbor, New York, 1988. If desired, the mRNA encoding the heavy and light chains may be isolated and mutagenized by cloning in *E. coli*, and the heavy and light
15 chains may be mixed to further enhance the affinity of the antibody.

 The antibodies find particular use in diagnostic assays for developmental abnormalities, basal cell carcinomas and other tumors associated with mutations in *ptc*. Staging, detection and typing of tumors may utilize a quantitative immunoassay for the presence or absence of normal *ptc*. Alternatively, the presence of mutated forms of *ptc* may be determined. A reduction in
20 normal *ptc* and/or presence of abnormal *ptc* is indicative that the tumor is *ptc*-associated.

 A sample is taken from a patient suspected of having a *ptc*-associated tumor, developmental abnormality or BCNS. Samples, as used herein, include biological fluids such as blood, cerebrospinal fluid, tears, saliva, lymph, dialysis fluid and the like- organ or tissue culture derived fluids, and fluids extracted from physiological tissues. Also included in the term are
25 derivatives and fractions of such fluids. Biopsy samples are of particular interest, e.g. skin lesions, organ tissue fragments, etc. Where metastasis is suspected, blood samples may be preferred. The number of cells in a sample will generally be at least about 10³, usually at least
10⁴ more usually at least about 10⁵. The cells may be dissociated, in the case of solid tissues,

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5 or tissue sections may be analyzed. Alternatively a lysate of the cells may be prepared.

Diagnosis may be performed by a number of methods. The different methods all determine the absence or presence of normal or abnormal *ptc* in patient cells suspected of having a mutation in *ptc*. For example, detection may utilize staining of intact cells or histological sections, performed in accordance with conventional methods. The antibodies of interest are
10 added to the cell sample, and incubated for a period of time sufficient to allow binding to the epitope, usually at least about 10 minutes. The antibody may be labeled with radioisotopes, enzymes, fluorescers, chemilumescers, or other labels for direct detection. Alternatively, a second stage antibody or reagent is used to amplify the signal. Such reagents are well-known in the art. For example, the primary antibody may be conjugated to biotin, with horseradish
15 peroxidase-conjugated avidin added as a second stage reagent. Final detection uses a substrate that undergoes a color change in the presence of the peroxidase. The absence or presence of antibody binding may be determined by various methods, including flow cytometry of dissociated cells, microscopy, radiography, scintillation counting, etc.

An alternative method for diagnosis depends on the *in vitro* detection of binding between
20 antibodies and *ptc* in a lysate. Measuring the concentration of *ptc* binding in a sample or fraction thereof may be accomplished by a variety of specific assays. A conventional sandwich type assay may be used. For example, a sandwich assay may first attach *ptc*-specific antibodies to an insoluble surface or support. The particular manner of binding is not crucial so long as it is compatible with the reagents and overall methods of the invention. They may be bound to the
25 plates covalently or non-covalently, preferably non-covalently.

The insoluble supports may be any compositions to which polypeptides can be bound, which is readily separated from soluble material, and which is otherwise compatible with the overall method. The surface of such supports may be solid or porous and of any convenient

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5 shape. Examples of suitable insoluble supports to which the receptor is bound include beads, e.g. magnetic beads, membranes and microtiter plates. These are typically made of glass, plastic (e.g. polystyrene), polysaccharides, nylon or nitrocellulose. Microtiter plates are especially convenient because a large number of assays can be carried out simultaneously, using small amounts of reagents and samples.

10 Patient sample lysates are then added to separately assayable supports (for example, separate wells of a microtiter plate) containing antibodies. Preferably, a series of standards, containing known concentrations of normal and/or abnormal *ptc* is assayed in parallel with the samples or aliquots thereof to serve as controls. Preferably, each sample and standard will be added to multiple wells so that mean values can be obtained for each. The incubation time
15 should be sufficient for binding, generally, from about 0.1 to 3 hr is sufficient. After incubation, the insoluble support is generally washed of non-bound components. Generally, a dilute non-ionic detergent medium at an appropriate pH, generally 7-8, is used as a wash medium. From one to six washes may be employed, with sufficient volume to thoroughly wash nonspecifically bound proteins present in the sample.

20 After washing, a solution containing a second antibody is applied. The antibody will bind *ptc* with sufficient specificity such that it can be distinguished from other components present. The second antibodies may be labeled to facilitate direct, or indirect quantification of binding. Examples of labels that permit direct measurement of second receptor binding include radiolabels, such as ³H or ¹²⁵I, fluorescers, dyes, beads, chemiluminescers, colloidal particles,
25 and the like. Examples of labels which permit indirect measurement of binding include enzymes where the substrate may provide for a colored or fluorescent product. In a preferred embodiment, the antibodies are labeled with a covalently bound enzyme capable of providing a detectable product signal after addition of suitable substrate. Examples of suitable enzymes

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5 for use in conjugates include horseradish peroxidase, alkaline phosphatase, malate dehydrogenase and the like. Where not commercially available, such antibody-enzyme conjugates are readily produced by techniques known to those skilled in the art. The incubation time should be sufficient for the labeled ligand to bind available molecules. Generally, from about 0.1 to 3 hr is sufficient, usually 1 hr sufficing.

10 After the second binding step, the insoluble support is again washed free of non-specifically bound material. The signal produced by the bound conjugate is detected by conventional means. Where an enzyme conjugate is used, an appropriate enzyme substrate is provided so a detectable product is formed.

Other immunoassays are known in the art and may find use as diagnostics. Ouchterlony
15 plates provide a simple determination of antibody binding. Western blots may be performed on protein gels or protein spots on filters, using a detection system specific for *ptc* as desired, conveniently using a labeling method as described for the sandwich assay.

Other diagnostic assays of interest are based on the functional properties of *ptc* protein itself. Such assays are particularly useful where a large number of different sequence changes
20 lead to a common phenotype, i.e., loss of protein function leading to oncogenesis or developmental abnormality. For example, a functional assay may be based on the transcriptional changes mediated by *hedgehog* and *patched* gene products. Addition of soluble Hh to embryonic stem cells causes induction of transcription in target genes. The presence of functional *ptc* can be determined by its ability to antagonize Hh activity. Other functional assays
25 may detect the transport of specific molecules mediated by *ptc*, in an intact cell or membrane fragment. Conveniently, a labeled substrate is used, where the transport in or out of the cell can be quantitated by radiography, microscopy, flow cytometry, spectrophotometry, etc. Other assays may detect conformational changes, or changes in the subcellular localization of *patched*

5 protein.

By providing for the production of large amounts of patched protein, one can identify ligands or substrates that bind to, modulate or mimic the action of *patched*. A common feature in basal cell carcinoma is the loss of adhesion between epidermal and dermal layers, indicating a role for *ptc* in maintaining appropriate cell adhesion. Areas of investigation include the
10 development of cancer treatments, wound healing, adverse effects of aging, metastasis, etc.

Drug screening identifies agents that provide a replacement for *ptc* function in abnormal cells. The role of *ptc* as a tumor suppressor indicates that agents which mimic its function, in terms of transmembrane transport of molecules, transcriptional down-regulation, etc., will inhibit the process of oncogenesis. These agents may also promote appropriate cell adhesion in wound
15 healing and aging, to reverse the loss of adhesion observed in metastasis, etc. Conversely, agents that reverse *ptc* function may stimulate controlled growth and healing. Of particular interest are screening assays for agents that have a low toxicity for human cells. A wide variety of assays may be used for this purpose, including labeled *in vitro* protein-protein binding assays, electrophoretic mobility shift assays, immunoassays for protein binding, and the like. The
20 purified protein may also be used for determination of three-dimensional crystal structure, which can be used for modeling intermolecular interactions, transporter function, etc.

The term "agent" as used herein describes any molecule, e.g. protein or pharmaceutical, with the capability of altering or mimicking the physiological function of *patched*. Generally a plurality of assay mixtures are run in parallel with different agent concentrations to obtain a
25 differential response to the various concentrations. Typically, one of these concentrations serves as a negative control, i.e. at zero concentration or below the level of detection.

Candidate agents encompass numerous chemical classes, though typically they are organic molecules, preferably small organic compounds having a molecular weight of more than

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5 50 and less than about 2,500 daltons. Candidate agents comprise functional groups necessary for structural interaction with proteins, particularly hydrogen bonding, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups. The candidate agents often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional
10 groups. Candidate agents are also found among biomolecules including peptides, saccharides, fatty 'ds, steroids, purines, pyrimidines, derivatives, structural analogs or a combinations thereof.

Candidate agents are obtained from a wide variety of sources including libraries of synthetic or natural compounds. For example, numerous means are available for random and directed synthesis of a wide variety of organic compounds and biomolecules, including
15 expression of randomized oligonucleotides and oligopeptides. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available or readily produced. Additionally, natural or synthetically produced libraries and compounds are readily modified through conventional chemical, physical and biochemical means, and may be used to produce combinatorial libraries. Known pharmacological agents may be subjected to directed
20 or random chemical modifications, such as acylation, alkylation, esterification, amidification, etc. to produce structural analogs.

Where the screening assay is a binding assay, one or more of the molecules may be joined to a label, where the label can directly or indirectly provide a detectable signal. Various labels include radioisotopes, fluorescers, chemilumescers, enzymes, specific binding molecules,
25 particles, e.g. magnetic particles, and the like. Specific binding molecules include pairs, such as biotin and streptavidin, digoxin and antidigoxin etc. For the specific binding members, the complementary member would normally be labeled with a molecule that provides for detection, in accordance with known procedures.

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5 A variety of other reagents may be included in the screening assay. These include reagents like salts, neutral proteins, e.g. albumin, detergents, etc that are used to facilitate optimal protein-protein binding and/or reduce nonspecific or background interactions. Reagents that improve the efficiency of the assay, such as protease inhibitors, nuclease inhibitors, anti-microbial agents, etc. may be used. The mixture of components are added in any order that
10 provides for the requisite binding. Incubations are performed at any suitable temperature, typically between 4° and 40° C. Incubation periods are selected for optimum activity, but may also be optimized to facilitate rapid high-throughput screening. Typically between 0.1 and 1 hours will be sufficient.

Other assays of interest detect agents that mimic patched function, such as repression
15 of target gene transcription, transport of patched substrate compounds, etc. For example, an expression construct comprising a *patched* gene may be introduced into a cell line under conditions that allow expression. The level of patched activity is determined by a functional assay, as previously described. In one screening assay, candidate agents are added in combination with a Hh protein, and the ability to overcome Hh antagonism of *ptc* is detected.
20 In another assay, the ability of candidate agents to enhance *ptc* function is determined. Alternatively, candidate agents are added to a cell that lacks functional *ptc*, and screened for the ability to reproduce *ptc* in a functional assay.

The compounds having the desired pharmacological activity may be administered in a physiologically acceptable carrier to a host for treatment of cancer or developmental
25 abnormalities attributable to a defect in *patched* function. The compounds may also be used to enhance patched function in wound healing, aging, etc. The inhibitory agents may be administered in a variety of ways, orally, topically, parenterally e.g. subcutaneously, intraperitoneally, by viral infection, intravascularly, etc. Topical treatments are of particular

5 interest. Depending upon the manner of introduction, the compounds may be formulated in a variety of ways. The concentration of therapeutically active compound in the formulation may vary from about 0.1-100 wt. %.

The pharmaceutical compositions can be prepared in various forms, such as granules, tablets, pills, suppositories, capsules, suspensions, salves, lotions and the like. Pharmaceutical
10 grade organic or inorganic carriers and/or diluents suitable for oral and topical use can be used to make up compositions containing the therapeutically-active compounds. Diluents known to the art include aqueous media, vegetable and animal oils and fats. Stabilizing agents, wetting and emulsifying agents, salts for varying the osmotic pressure or buffers for securing an adequate pH value, and skin penetration enhancers can be used as auxiliary agents.

15 The gene or fragments thereof may be used as probes for identifying the 5' non-coding region comprising the transcriptional initiation region, particularly the enhancer regulating the transcription of *patched*. By probing a genomic library, particularly with a probe comprising the 5' coding region, one can obtain fragments comprising the 5' non-coding region. If necessary, one may walk the fragment to obtain further 5' sequence to ensure that one has at least a
20 functional portion of the enhancer. It is found that the enhancer is proximal to the 5' coding region, a portion being in the transcribed sequence and downstream from the promoter sequences. The transcriptional initiation region may be used for many purposes, studying embryonic development, providing for regulated expression of *patched* protein or other protein of interest during embryonic development or thereafter, and in gene therapy.

25 The gene may also be used for gene therapy. Vectors useful for introduction of the gene include plasmids and viral vectors. Of particular interest are retroviral-based vectors, e.g. moloney murine leukemia virus and modified human immunodeficiency virus- adenovirus vectors, etc. Gene therapy may be used to treat skin lesions, an affected fetus, etc., by

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5 transfection of the normal gene into embryonic stem cells or into other fetal cells. A wide variety of viral vectors can be employed for transfection and stable integration of the gene into the genome of the cells. Alternatively, micro-injection may be employed, fusion, or the like for introduction of genes into a suitable host cell. See, for example, Dhawan *et al.* (1991) *Science* 254:1509-1512 and Smith *et al.* (1990) *Molecular and Cellular Biology* 3268-3271.

10 The following examples are offered by illustration not by way of limitation.

EXPERIMENTAL

Methods and Materials

PCR on Mosquito (Anopheles gambiae) Genomic DNA. PCR primers were based on amino acid stretches of fly *ptc* that were not likely to diverge over evolutionary time and were
15 of low degeneracy. Two such primers (P2R1 (SEQ ID NO:14)-
GGACGAATTCAARGTNCAYCARYTNTGG, P4R1: (SEQ ID NO:15)
GGACGAATTCCYTCCCARAARCANTC, (the underlined sequences are Eco RI linkers)
amplified an appropriately sized band from mosquito genomic DNA using the PCR. The
program conditions were as follows:

20 94°C 4 min.; 72°C Add Taq;
[49°C 30 sec.; 72°C 90 sec.; 94°C 15 sec] 3 times
[94°C 15 sec.; 50°C 30 sec.; 72°C 90 sec] 35 times
72 °C 10 min; 4°C hold

25 This band was subcloned into the EcoRV site of pBluescript II and sequenced using the USB
Sequence kit.

Screen of a Butterfly cDNA Library with Mosquito PCR Product. Using the mosquito
PCR product (SEQ ID NO:7) as a probe, a 3 day embryonic *Precis coenia* λ gt10 cDNA library
(generously provided by Sean Carroll) was screened. Filters were hybridized at 65° C overnight
30 in a solution containing 5xSSC, 10% dextran sulfate, 5x Denhardt's, 200 μ g/ml sonicated

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5 salmon sperm DNA, and 0.5% SDS. Filters were washed in 0.1X SSC, 0.1% SDS at room temperature several times to remove nonspecific hybridization. Of the 100,000 plaques initially screened, 2 overlapping clones, L1 and L2, were isolated, which corresponded to the N terminus of butterfly *ptc*. Using L2 as a probe, the library filters were rescreened and 3 additional clones (L5, L7, L8) were isolated which encompassed the remainder of the *ptc* coding sequence. The
10 full length sequence of butterfly *ptc* (SEQ ID NO:3) was determined by ABI automated sequencing.

Screen of a Tribolium (beetle) Genomic Library with Mosquito PCR Product and 900 bp Fragment from the Butterfly Clone. A λ gem11 genomic library from *Tribolium castaneum* (gift of Rob Dennell) was probed with a mixture of the mosquito PCR (SEQ ID NO:7) product
15 and BstXI/EcoRI fragment of L2. Filters were hybridized at 55° C overnight and washed as above. Of the 75,000 plaques screened, 14 clones were identified and the SacI fragment of T8 (SEQ ID NO:1), which crosshybridized with the mosquito and butterfly probes, was subcloned into pBluescript.

PCR on Mouse cDNA Using Degenerate Primers Derived from Regions Conserved in
20 *the Four Insect Homologues.* Two degenerate PCR primers (P4REV- (SEQ ID NO:16) GGACGAATTCYTNGANTGYTTTGTGGGA- P22- (SEQ ID NO:17) CATACCAGCCAAGCTTGTCTGCGCCARTGCAT) were designed based on a comparison of *ptc* amino acid sequences from fly (*Drosophila melanogaster*) (SEQ ID NO:6), mosquito (*Anopheles gambiae*) (SEQ ID NO:8), butterfly (*Precis coenia*) (SEQ ID NO:4), and beetle (*Tribolium castaneum*)
25 (SEQ ID NO:2). I represents inosine, which can form base pairs with all four nucleotides. P22 was used to reverse transcribe RNA from 12.5 dpc mouse limb bud (gift from David Kingsley) for 90 min at 37° C. PCR using P4REV (SEQ ID NO:17) and P22 (SEQ ID NO:18) was then performed on 1 μ l of the resultant cDNA under the following conditions:

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- 5 94°C 4 min.; 72°C Add Taq;
 [94 °C 15 sec.- 50 °C 30 sec.- 72 °C 90 sec.] 35 times
 72 °C 10 min.-, 4 °C hold

PCR products of the expected size were subcloned into the TA vector (Invitrogen)
10 and sequenced with the Sequenase Version 2.0 DNA Sequencing Kit (U. S. B.).

Using the cloned mouse PCR fragment as a probe, 300,000 plaques of a mouse 8.5 dpc
λgt10 cDNA library (a gift from Brigid Hogan) were screened at 65° C as above and washed
in 2x SSC, 0.1% SDS at room temperature. 7 clones were isolated, and three (M2, M4, and
M8) were subcloned into pBluescript II. 200,000 plaques of this library were rescreened using
15 first, a 1.1 kb EcoRI fragment from M2 to identify 6 clones (M9-M16) and secondly a mixed
probe containing the most N terminal (XhoI fragment from M2) and most C terminal sequences
(BamHI/BglIII fragment from M9) to isolate 5 clones (M17-M21). M9, M10, M14, and M17-
21 were subcloned into the EcoRI site of pBluescript II (Stratagene).

RNA Blots and in situ Hybridizations in Whole and Sectioned Mouse Embryos:

- 20 *Northern.* A mouse embryonic Northern blot and an adult multiple tissue Northern blot
(obtained from Clontech) were probed with a 900 bp EcoRI fragment from an N terminal coding
region of mouse *ptc*. Hybridization was performed at 65° C in 5x SSPE, 10x Denhardt's, 100
μg/ml sonicated salmon sperm DNA, and 2% SDS. After several short room temperature
washes in 2x SSC, 0.05% SDS, the blots were washed at high stringency in 0.1 X SSC, 0.1%
25 SDS at 50° C.

In situ hybridization of sections: 7.75, 8.5, 11.5, and 13.5 dpc mouse embryos were
dissected in PBS and frozen in Tissue-Tek medium at -80° C. 12-16 μm frozen sections were
cut, collected onto VectaBond (Vector Laboratories) coated slides, and dried for 30-60 minutes
at room temperature. After a 10 minute fixation in 4% paraformaldehyde in PBS, the slides

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5 were washed 3 times for 3 minutes in PBS, acetylated for 10 minutes in 0.25% acetic anhydride in triethanolamine, and washed three more times for 5 minutes in PBS. Prehybridization (50% formamide, 5X SSC, 250 µg/ml yeast tRNA, 500 µg/ml sonicated salmon sperm DNA, and 5x Denhardt's) was carried out for 6 hours at room temperature in 50% formamide/5x SSC humidified chambers. The probe, which consisted of 1 kb from the N-terminus of *ptc*, was
10 added at a concentration of 200-1000 ng/ml into the same solution used for prehybridization, and then denatured for five minutes at 80° C. Approximately 75 µl of probe were added to each slide and covered with Parafilm. The slides were incubated overnight at 65° C in the same humidified chamber used previously. The following day, the probe was washed successively in 5X SSC (5 minutes, 65° C), 0.2X SSC (1 hour, 65° C), and 0.2X SSC (10 minutes, room
15 temperature). After five minutes in buffer B1 (0.1M maleic acid, 0.15 M NaCl, pH 7.5), the slides were blocked for 1 hour at room temperature in 1% blocking reagent (Boehringer-Mannheim) in buffer B1, and then incubated for 4 hours in buffer B1 containing the DIG-AP conjugated antibody (Boehringer-Mannheim) at a 1:5000 dilution. Excess antibody was removed during two 15 minute washes in buffer B1, followed by five minutes in buffer B3 (100
20 mM Tris, 100mM NaCl, 5mM MgCl₂, pH 9.5). The antibody was detected by adding an alkaline phosphatase substrate (350 µl 75 mg/ml X-phosphate in DMF, 450 µl 50 mg/ml NBT in 70% DMF in 100 mls of buffer B3) and allowing the reaction to proceed overnight in the dark. After a brief rinse in 10 mM Tris, 1mM EDTA, pH 8.0, the slides were mounted with Aquamount (Lerner Laboratories).

25 *Drosophila 5-transcriptional initiation region β-gal constructs.* A series of constructs were designed that link different regions of the *ptc* promoter from *Drosophila* to a LacZ reporter gene in order to study the cis regulation of the *ptc* expression pattern. See Fig. 1. A 10.8kb BamHI/BspMI fragment comprising the 5'-non-coding region of the mRNA at its 3'-

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5 terminus was obtained and truncated by restriction enzyme digestion as shown in Fig. 1. These expression cassettes were introduced into *Drosophila* lines using a P-element vector (Thummel *et al.* (1988) Gene 74:445-456), which were injected into embryos, providing flies which could be grown to produce embryos. (See Spradling and Rubin (1982) Science 218:341-347 for a description of the procedure.) The vector used a pUC8 background into which was introduced
10 the white gene to provide for yellow eyes, portions of the P-element for integration, and the constructs were inserted into a polylinker upstream from the LacZ gene. The resulting embryos, larvae, and adults were stained using antibodies to LacZ protein conjugated to HRP and the samples developed with OPD dye to identify the expression of the LacZ gene. The staining pattern in embryos is described in Fig. 1, indicating whether there was staining during the early
15 and late development of the embryo.

Isolation of a Mouse ptc Gene. Homologues of fly *ptc* (SEQ ID NO:6) were isolated from three insects: mosquito, butterfly and beetle, using either PCR or low stringency library screens. PCR primers to six amino acid stretches of *ptc* of low mutability and degeneracy were designed. One primer pair, P2 and P4, amplified an homologous fragment of *ptc* from
20 mosquito genomic DNA that corresponded to the first hydrophilic loop of the protein. The 345bp PCR product (SEQ ID NO:7) was subcloned and sequenced and when aligned to fly *ptc*, showed 67% amino acid identity.

The cloned mosquito fragment was used to screen a butterfly λ gt 10 cDNA library. Of 100,000 plaques screened, five overlapping clones were isolated and used to obtain the full
25 length coding sequence. The butterfly *ptc* homologue (SEQ ID NO:4) is 1,311 amino acids long and overall has 50% amino acid identity (72% similarity) to fly *ptc*. With the exception of a divergent C-terminus, this homology is evenly spread across the coding sequence. The mosquito PCR clone (SEQ ID NO:7) and a corresponding fragment of butterfly cDNA were

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5 used to screen a beetle *λ*gem11 genomic library. Of the plaques screened, 14 clones were identified. A fragment of one clone (T8), which hybridized with the original probes, was subcloned and sequenced. This 3kb piece contains an 89 amino acid exon (SEQ ID NO:2) which is 44% and 51% identical to the corresponding regions of fly and butterfly *ptc* respectively.

10 Using an alignment of the four insect homologues in the first hydrophilic loop of the *ptc*, two PCR primers were designed to a five and six amino acid stretch which were identical and of low degeneracy. These primers were used to isolate the mouse homologue using RT-PCR on embryonic limb bud RNA. An appropriately sized band was amplified and upon cloning and sequencing, it was found to encode a protein 65% identical to fly *ptc*. Using the cloned PCR
15 product and subsequently, fragments of mouse *ptc* cDNA, a mouse embryonic *λ*cDNA library was screened. From about 300,000 plaques, 17 clones were identified and of these, 7 form overlapping cDNA's that comprise most of the protein-coding sequence (SEQ ID NO:9).

Developmental and Tissue Distribution of Mouse ptc RNA. In both the embryonic and adult Northern blots, the *ptc* probe detects a single 8kb message. Further exposure does not
20 reveal any additional minor bands. Developmentally, *ptc* mRNA is present in low levels as early as 7 dpc and becomes quite abundant by 11 and 15 dpc. While the gene is still present at 17 dpc, the Northern blot indicates a clear decrease in the amount of message at this stage. In the adult, *ptc* RNA is present in high amounts in the brain and lung, as well as in moderate amounts in the kidney and liver. Weak signals are detected in heart, spleen, skeletal muscle, and testes.

25 *In situ Hybridization of Mouse ptc in Whole and Section Embryos.* Northern analysis indicates that *ptc* mRNA is present at 7 dpc, while there is no detectable signal in sections from 7.75 dpc embryos. This discrepancy is explained by the low level of transcription. In contrast, *ptc* is present at high levels along the neural axis of 8.5 dpc embryos. By 11.5 dpc, *ptc* can be

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5 detected in the developing lung buds and gut, consistent with its adult Northern profile. In addition, the gene is present at high levels in the ventricular zone of the central nervous system, as well as in the zona limitans of the prosencephalon. *ptc* is also strongly transcribed in the condensing cartilage of 11.5 and 13.5 dpc limb buds, as well as in the ventral portion of the somites, a region which is prospective sclerotome and eventually forms bone in the vertebral
10 column. *ptc* is present in a wide range of tissues from endodermal, mesodermal and ectodermal origin supporting its fundamental role in embryonic development.

Isolation of the Human ptc Gene. To isolate human *ptc* (*hptc*), 2×10^5 plaques from a human lung cDNA library (HL3022a, Clontech) were screened with a 1kbp mouse *ptc* fragment, M2-2. Filters were hybridized overnight at reduced stringency (60° C in 5X SSC,
15 10% dextran sulfate, 5X Denhardt's, 0.2 mg/ml sonicated salmon sperm DNA, and 0.5% SDS). Two positive plaques (H1 and H2) were isolated, the inserts cloned into pBluescript, and upon sequencing, both contained sequence highly similar to the mouse *ptc* homolog. To isolate the 5' end, an additional 6×10^5 plaques were screened in duplicate with M2-3 EcoRI and M2-3 Xho I (containing 5' untranslated sequence of mouse *ptc*) probes. Ten plaques were purified
20 and of these, inserts were subcloned into pBluescript. To obtain the full coding sequence, H2 was fully and H14, H20, and H21 were partially sequenced. The 5.1kbp of human *ptc* sequence (SEQ ID NO:18) contains an open reading frame of 1447 amino acids (SEQ ID NO:19) that is 96% identical and 98% similar to mouse *ptc*. The 5' and 3' untranslated sequences of human *ptc* (SEQ ID NO:18) are also highly similar to mouse *ptc* (SEQ ID NO:19) suggesting
25 conserved regulatory sequence.

Comparison of Mouse, Human, Fly and Butterfly Sequences. The deduced mouse *ptc* protein sequence (SEQ ID NO:10) has about 38% identical amino acids to fly *ptc* over about 1,200 amino acids. This amount of conservation is dispersed through much of the protein

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5 excepting the C-terminal region. The mouse protein also has a 50 amino acid insert relative to the fly protein. Based on the sequence conservation of *ptc* and the functional conservation of *hedgehog* between fly and mouse, one concludes that *ptc* functions similarly in the two organisms. A comparison of the amino acid sequences of mouse (*mptc*) (SEQ ID NO:10), human (*hptc*) (SEQ ID NO:19), butterfly (*bptc*) (SEQ ID NO:4) and *drosophila* (*ptc*) (SEQ ID NO:6) is shown in Table 1.

TABLE 1

ALIGNMENT OF HUMAN, MOUSE, FLY, AND BUTTERFLY PTC HOMOLOGS

HPTC	MASAGHAAEPQDR--CGGGSGCIGAPGRPACGGRRRTGGLRRAAAPDRDYLRPSYCD
MPTC	MASAGHAA-----GALGRQAGCGRRRTGCPHRA-APDRDYLRPSYCD
15 PTC	M-----DRDSLPRVPDTHGD--VVDE-----KLFSDL-----YI-RTSWVDA
BPTC	MVAPDSEAPSNPRITAAHESPCATEA-----RHSADL-----YI-RTSWVDA
	* * *
HPTC	AFALQISKQKATGRKAPLWLRAPQRLLFKLGCIYQKNCCKFLVVGLLIFGAFVGLKA
20 MPTC	AFALQISKQKATGRKAPLWLRAPQRLLFKLGCIYQKNCCKFLVVGLLIFGAFVGLKA
PTC	QVALDQIDKQKARGSRATYLRVFSQSHLETLGSSVQKHAGKVLVAILVLSTPCVGLKS
BPTC	ALALSELEKGNIEGGRTSLWIRAWLQEQFLILGCFLQGDAGKVLVAILVLSTPCVGLKS
	** * * *
25 HPTC	ANLETNVEELWVEVGGVRSRELNYTRQKIGEEAMFNPQLMIQTPKEEGANVLTTEALLQH
MPTC	ANLETNVEELWVEVGGVRSRELNYTRQKIGEEAMFNPQLMIQTPKEEGANVLTTEALLQH
PTC	AQIHSKVEQLWVQEGGRLEAELAYTKTIGEDSATHQLLIQTTHDPNASVLHPQALLAH
BPTC	AQIHTRVQQLWVQEGGRLEAELKYTAQALGEADSSSTHQLVIQAKDPDVSLLHPGALLER
	* * * *
30 HPTC	LDSALQASRVHVYMYNRQWLEHLCKYKSGELITET-GYMDQIIIEYLYPCLIIITPLDCFWE
MPTC	LDSALQASRVHVYMYNRQWLEHLCKYKSGELITET-GYMDQIIIEYLYPCLIIITPLDCFWE
PTC	LEVLVKATAVKVHLYDTWGLRDHCNMPSTPSFEGIIYIEQILRHLIPCSIITPLDCFWE
BPTC	LKVVAATRTVTVMYDIEWLKDLCYSPSIPDFEGYHHIESIIDNVIPCAIITPLDCFWE
35	* * * *
HPTC	GAKLQSGTAYLLGKPLR----WTNFDPLEFLEELK-----KINYQVDSWEEMLNKAEV
MPTC	GAKLQSGTAYLLGKPLR----WTNFDPLEFLEELK-----KINYQVDSWEEMLNKAEV
PTC	GSQLL-GFESAVVPIPLNQRLWTTLNPAVSVQYMKQKMSSEKISDFETVEQYMKRAAI
40 BPTC	GSQLL-GPDYPIYVPHLKHKLQWTHLNPLEVVEVK-KL---KFQFPLSTIEAYMKRAGI
	* * * *
HPTC	GHCYMDRPCLNPAFDCPATAPNKNSTKPLDMALVLNCGGCHLSRKYMHWQELIVGGTV
MPTC	GHCYMDRPCLNPAFDCPATAPNKNSTKPLDVALVLNCGGCGLSRKYMHWQELIVGGTV
45 PTC	GSGYMKPCLNPLNPNCPDAPNKNSTQPPDVGAILSGGCYCYAAKHGHWPEELIVGGRK
BPTC	TSAYMKPCLDPTDPHCPATAPNKNKSGHIPDVAELSHGCYGFAAAYMHWPQELIVGGAT
	** * * *
HPTC	INSGELVAAALQTMPLMTPRQMYEHFPGCYEVVSHINWEDRAAAILRAWQRTYVEV
MPTC	INATGELVAAALQTMPLMTPRQMYEHFPGCYEVVSHINWEDRAAAILRAWQRTYVEV

[illegible]

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5 HPTC N1RPHRFEWVHDKADYMPETRLRI PAAEFIEYAQFFPYLNGLRDTSDFVZAEIKVRTICS
MPTC N1RPHRFEWVHDKADYMPETRLRI PAAEFIEYAQFFPYLNGLRDTSDFVZAEIKVVRVICS
PTC KLYPEPRQYTFHQFNEY----DLKIPKSLPLVYAQMPPYLBGLTDTSQIKTLIGHIRDLSV
BPTC NLKQPQQRNIHSPKDV----HLEIKKSSPLIXTQLPFYLSGLSDTDSIKTLIRASVRDLCL

10 HPTC NYTSLGGLSSYPNGYFFLFWEQYIGLPHWLLLFISVVLACTFLVCAVFLNPNWTAGIIVMV
MPTC NYTSLGGLSSYPNGYFFLFWEQYISLRHWWLLLSISVVLACTFLVCAVFLNPNWTAGIIVMV
PTC KYEGFGLPNYPSPGIPPIFWEQYMTLRSSLAMILACVLLAALVLSLSSVNAAVLVLS
BPTC KYEAKGLPNFPPEGIPFLFWEQYLYLRTSLLLALACALGAVFIAVMVLLNNAVAVLVTLA

15 HPTC LALMTVELFGHMLGIGIKLSAVPVVILIASVIGVEFTVHVALAFLTAIGDKNRRRAVLAL
MPTC LALMTVELFGHMLGIGIKLSAVPVVILIASVIGVEFTVHVALAFLTAIGDKNRRRAVLAL
PTC VLASLAQIFGAMTLLGIKLSAIPAVILISVGMMLCFNVILISLGFMTSVGNRQRRVQLSM
BPTC LATLVQLLGLVHALLGVKLSAMPVVLVLAIGRGVHTVHLCLOFVTSIGCKRRRASLAL

20 HPTC EHMFAFVLDGAVSTLLGVLNLGASEDFIVRYFFAVLAILTILGVLNGLVLLPVLLSFPFQ
MPTC EHMFAFVLDGAVSTLLGVLNLGASEDFIVRYFFAVLAILTVLGVNGLVLLPVLLSFPFQ
PTC QMSLGPLVHGMLTSGVAVFMLSTSPPEFVIPHFCVLLVVLVLCVGCNLSLVFPILLSMVG
BPTC ESVLAPVVHGLAALAAASMLA. ASEFGFVARLFLRLLLALVFLGLIDGLLFFPIVLSILO

25 HPTC PYPEVSPANGLNRLPTSPPEPPSVVRPAMPFGHTHSGSDSSDSEYSSQTTVSGLSE-EL
MPTC PCPEVSPANGLNRLPTSPPEPPSVVRPAMPFGHTHSGSDSSDSEYSSQTTVSGLSE-EL
PTC PEAKLVPLEHPDRISTPSPLPVRSSSKRSKSYVVGQSRSSRGSCQKSHHHHKLNDPDL
BPTC PAAEVRPIEHPERLSTSPKPCSP IHPKRSSSSSGGDKSRTS--KSAPRPC----APSL

35 HPTC RHYEAOQQAGGPAHQVIVEATENPVFAHSTVVHPSRHHPPSNPRQQPHLDSGSLPPGRQ
MPTC RQYEAOQQAGGPAHQVIVEATENPVFAHSTVVHPSRHHPPSNPRQQPHLDSGSLPPGRQ
PTC TTITEEPQSWKSSNSSIQMPNDWTYQFREQ--RPASYAAPPYAYHKAQAQHQHQGPFT
BPTC TTITEEPSSWHSSAHSVQSSMQSIVVQPEVVVETTTYNQSDSASGRSTPTKSSHGCAITT

40 HPTC GQQPRRDPFREGWLPPLYRPRRDAFEISTECHSGPSNRARWCPGARSHNPPNPASTAMG
MPTC GQQPRRDPFREGWLPPPYRPRRDAFEISTECHSGPSNRDRSGPGARSHNPRNPSTAMG
PTC TFPFPFPTA-----YPPQLSIVVQPEVTVETTHS-----DS
BPTC TKVTATANIKVEVVTSPDRKSRRSYHYDRDRDRDEDNRDRDRDRDRDRDRDRDRDRDRDR

45 HPTC SSVPGYQCPITTTVTSASVTVAVHPPVPVPGPCRNPRGGLCPGY---PETDHGLFEDPHVP
MPTC SSVPSYQCPITTTVTSASVTVAVHPP---PGPCRNPRGGPCGYESYPETDHGVFEDPHVP
PTC NT-----TKVTATANIKVELAMP-----CPAVRS---YNFT6-----
BPTC DR-----DRERSRERDRP.DRYRD-----EPDHPA---SPRENGRDSGHE-----

50 HPTC FHVRCERRDSKVEVIELQDVECEERPRGSSSN
MPTC FHVRCERRDSKVEVIELQDVECEERPWGSSSN
PTC -----
BPTC -----SDSSRH

55 HPTC
MPTC
PTC
BPTC

The identity of ten other clones recovered from the mouse library is not determined.

These cDNAs cross-hybridize with mouse *ptc* sequence, while differing as to their restriction

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5 maps. These genes encode a family of proteins related to the patched protein. Alignment of the human and mouse nucleotide sequences, which includes coding and noncoding sequence, reveals 89% identity.

Radiation hybrid mapping of the human ptc gene. Oligonucleotide primers and conditions for specifically amplifying a portion of the human *ptc* gene from genomic DNA by
10 the polymerase chain reaction were developed. This marker was designated STS SHGC-8725. It generates an amplification product of 196 bp, which is observed by agarose gel electrophoresis when a human DNA is used as a template, but not when rodent DNA is used. Samples were scored in duplicate for the presence or absence of the 196 bp product in 83 radiation hybrid DNA samples from the Stanford G3 Radiation Hybrid Panel (purchased from
15 Research Genetics, Inc.) By comparison of the pattern of G3 panel scores for those with a series of Genethon meiotic linkage 5 markers, it was determined that the human *ptc* gene had a two point lod score of 1,000 with the meiotic marker D9S287, based on no radiation breaks being observed between the gene and the marker in 83 hybrid cell lines. These results indicate that the *ptc* gene lies within 50-100 kb of the marker. Subsequent physical mapping in YAC and
20 BAC clones confirmed this close linkage estimate. Detailed map information can be obtained from <http://www.shgc.stanford.edu>.

Analysis of BCNS mutations. The basal cell nevus syndrome has been mapped to the same region of chromosome 9q as was found for *ptc*. An initial screen of EcoRI digested DNA from probands of 84 BCNS kindreds did not reveal major rearrangements of the *ptc* gene, and
25 so screening was performed for more subtle sequence abnormalities. Using vectorette PCR, by the method according to Riley *et al.* (1990) N.A.R. 18:2887-2890, on a BAC that contains genomic DNA for the entire coding region of *ptc*, the intronic sequence flanking 20 of the 24 exons was determined. Single strand conformational polymorphism analysis of PCR-amplified

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5 DNA from normal individuals, BCNS o patients and sporadic basal cell carcinomas (BCC) was performed for 20 exons of *ptc* coding sequence. The amplified samples giving abnormal bands on SSCP were then sequenced.

In blood cell DNA from BCNS individuals, four independent sequence changes were found; two in exon 15 and two in exon 10. One 49 year old man was found to have a sequence
10 change in exon 15. His affected sister and daughter have the same alteration, but three unaffected relatives do not. His blood cell DNA has an insertion of 9 base pairs at nucleotide 2445 of the coding sequence, resulting in the insertion of three amino acids (PNI) after amino acid 815. Because the normal sequence preceding the insertion is also PNI, a direct repeat has been formed.

15 The second case of an exon 15 change is an 18 year old woman who developed jaw cysts at age 9 and BCCs at age 6. The developmental effects together with the BCCs indicate that she has BCNS, although none of her relatives are known to have the syndrome. Her blood cell DNA has a deletion of 11 bp, removing the sequence ATATCCAGCAC at nucleotides 2441 to 2452 of the coding sequence. In addition, nucleotide 2452 is changed from a T to an A. The
20 deletion results in a frameshift that is predicted to truncate the protein after amino acid 813 with the addition of 9 amino acids. The predicted mutant protein is truncated after the seventh transmembrane domain. In *Drosophila*, a *ptc* protein that is truncated after the sixth transmembrane domain is inactive when ectopically expressed, in contrast to the full-length protein, suggesting that the human protein is inactivated by the exon 15 sequence change. The
25 patient with this mutation is the first affected family member, since her parents, age 48 and 50, have neither BCCs nor other signs of the BCNS- DNA from both parents' genes have the normal nucleotide sequence for exon 15, indicating that the alteration in exon 15 arose in the same generation as did the BCNS phenotype. Hence her disease is the result of a new mutation. This

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5 sequence change is not detected in 84 control chromosomes.

Analysis of sporadic basal cell carcinomas. To determine whether *ptc* is also involved in BCCs that are not associated with the BCNS or germline changes, DNA was examined from 12 sporadic BCCs. Three alterations were found in these tumors. In one tumor, a C to T transition in exon 3 at nucleotide 523 of the coding sequence changes a highly
10 conserved leucine to phenylalanine at residue 175 in the first putative extracellular loop domain. Blood cell DNA from the same individual does not have the alteration, suggesting that it arose somatically in the tumor. SSCP was used to examine exon 3 DNA from 60 individuals who do not have BCNS, and found no changes from the normal sequence. Two other sporadic BCCs have deletions encompassing exon 9 but not extending to exon 8.

15 The existence of sporadic and hereditary forms of BCCs is reminiscent of the characteristics of the two forms of retinoblastoma. This parallel, and the frequent deletion in tumors of the copy of chromosome 9q predicted by linkage to carry the wild-type allele, demonstrates that the human *ptc* is a tumor suppressor gene. *ptc* represses a variety of genes, including growth factors, during *Drosophila* development and may have the same effect in
20 human skin. The often reported large body size of BCNS patients also could be due to reduced *ptc* function, perhaps due to loss of control of growth factors. The C to T transition identified in *ptc* in the sporadic BCC is also a common genetic change in the *p53* gene in BCC and is consistent with the role of sunlight in causing these tumors. By contrast, the inherited deletion and insertion mutations identified in BCNS patients, as expected, are not those characteristic
25 of ultraviolet mutagenesis.

The identification of the *ptc* mutations as a cause of BCNS links a large body of developmental genetic information to this important human disease. In embryos lacking *ptc* function part of each body segment is transformed into an anterior-posterior mirror-image

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5 duplication of another part. The patterning changes in *ptc* mutants are due in part to derepression of another segment polarity gene, *wingless*, a homolog of the vertebrate Wnt genes that encodes secreted signaling proteins. In normal embryonic development, *ptc* repression of *wg* is relieved by the Hh signaling protein, which emanates from adjacent cells in the posterior part of each segment. The resulting localized *wg* expression in each segment primordium
10 organizes the pattern of bristles on the surface of the animal. The *ptc* gene inactivates its own transcription, while Hh signaling induces *ptc* transcription.

In flies two other proteins work together with Hh to activate target genes: the ser/thr kinase *fused* and the zinc finger protein encoded by *cubitus interruptus*. Negative regulators working together with *ptc* to repress targets are *protein kinase A* and *costal2*. Thus, mutations
15 that inactivate human versions of *protein kinase A* or *costal2*, or that cause excessive activity of human *hh*, *gli*, or a *fused* homolog, may modify the BCNS phenotype and be important in tumorigenesis.

In accordance with the subject invention, mammalian patched genes, including the mouse and human genes, are provided, which can serve many purposes. Mutations in the gene
20 are found in patients with basal cell nevus syndrome, and in sporadic basal cell carcinomas. The autosomal dominant inheritance of BCNS indicates that *patched* is a tumor suppressor gene. The patched protein may be used in a screening for agonists and antagonists, and for assaying for the transcription of *ptc* mRNA. The protein or fragments thereof may be used to produce antibodies specific for the protein or specific epitopes of the protein. In addition, the gene may
25 be employed for investigating embryonic development, by screening fetal tissue, preparing transgenic animals to serve as models, and the like.

As described above, patients with basal cell nevus syndrome have a high incidence of multiple basal cell carcinomas, medulloblastomas, and meningiomas. Because somatic *ptc*

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5 mutations have been found in sporadic basal cell carcinomas, we have screened for *ptc* mutations in several types of sporadic extracutaneous tumors. We found that 2 of 14 sporadic medulloblastomas bear somatic nonsense mutations in one copy of the gene and also deletion of the other copy. In addition, we identified mis-sense mutations in *ptc* in two of seven breast carcinomas, one of nine meningiomas, and one colon cancer cell line. No *ptc* gene mutations
10 were detected in 10 primary colon carcinomas and eighteen bladder carcinomas.

BCNS³ (OMIM #109400) is a rare autosomal dominant disease with diverse phenotypic abnormalities, both tumorous (BCCs, medulloblastomas, and meningiomas) and developmental (misshapen ribs, spina bifida occulta, and skull abnormalities; Gorlin, R.J.(1987) *Medicine* 66:98-113). The BCNS gene was mapped to chromosome 9q22.3 by linkage analysis
15 of BCNS families and by LOH analysis in sporadic BCCs (Gallani, M.R. *et al.* (1992) *Cell* 69:111-117). LOH in sporadic medulloblastomas has been reported in the same chromosome region (Schofield, D. *et al.* (1995) *Am J Pathol* 146:472-480). Recently, the human homologue of the *Drosophila patched* (PTCII) gene has been mapped to the BCNS region (Hahn, H. *et al.* (1996) *Cell* 85:841-851; Johnson, R.L. *et al.* (1996) *Science* 272:1668-1671; Gallani, M.R. *et al.* (1996) *Nat Genet* 14:78-81; Xie, J. *et al.* (1997) *Genes Chromosomes Cancer* 18:305-309),
20 and mutations in this gene have been found in the blood DNA of BCNS patients and in the DNA of sporadic BCCs (Hahn, H. *et al.*, *supra*; Johnson, R.L. *et al.*, *supra*; Gallani, M.R. *et al.*, *supra*; and Chidambaram, A. *et al.* (1996) *Cancer Res* 56:4599-4601). *ptc* appears to function as a tumor suppressor gene; inactivation abrogates its normal inhibition of the hedgehog
25 signaling pathway. Because of the wide variety of tumors in patients with the BCNS and wide tissue distribution of *ptc* gene expression, we have begun screening for *ptc* gene mutations in several types of human cancer, especially those present in increased numbers in BCNS patients (medulloblastomas), those in tissues derived embryologically from epidermis (breast carcinomas)

5 and those with chromosome 9q LOG (bladder carcinomas; see Cairns, P. *et al.* (1993) *Cancer Res* 53:1230-1232; and Sidransky, D. *et al.* (1997) *NEJM* 326:737-740).

Materials and methods

Clinical Materials. Diagnoses of all tumors were confirmed histologically. Cell lines were obtained from the America Type Culture Collection. DNA was extracted from tumors or
10 matched normal tissue (peripheral blood leukocytes or skin) as described (Cogen, P.H. *et al.* (1990) *Genomics* 8:279-285; and Sambrook, J. *et al.* Molecular Cloning: A Laboratory Manual, Ed. 2, Vol. 2, pp. 9.17 - 9.19, Cold Spring Harbor, NY (1989)).

PCR and Heteroduplex Analysis. PCR amplification and heteroduplex/SSCP analysis were performed as described (Johnson, R.L. *et al.*, *supra*; Spritz, R.A. *et al.* (1992) *Am J Hum*
15 *Genet* 51:1058-1065). Primers used and intron/exon boundary sequences of the *ptc* gene were derived as reported previously (Johnson, R.L. *et al.*, *supra*) and are shown in Table 1. Primers for exon 1 and 2 were from Hahn *et al.* (*supra*).

Sequence Analysis. Exon segments exhibiting bands were reamplified and were sequenced directly using the Sequenase sequencing kit according to the protocol recommended
20 by the manufacturer (United States Biochemical Corp.). A second sequencing was performed using independently amplified PCR products to confirm the sequence change. The amplified PCR products from each tumor were also cloned into the plasmid vector pCR 2.1 (InVitrogen), followed by sequence analysis of at least four independent clones. The sequence alteration was confirmed from at least two independent clones. Simplified amplification of specific allele
25 analysis was performed according to Lei and Hall (Lei, X. and Hall, B.G. (1994) *Biotechniques* 16:44-45).

Allele Loss Analysis. Microsatellites used for allelic loss analysis were D9S109, DpS119, D9S127, D9S196, and D9S287 described in the CHLC human screening set (Research

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5 Genetics): A part of the *ptc* intron 1 sequence was tested for polymorphism in a control population and found to be polymorphic in 80% of the samples tested. This microsatellite was used for analysis of *ptc* gene allelic loss in bladder carcinomas. The primer sequences are as follows: forward primer, 5'-CTGAGCAGATTTCCTCAGGTC-3'; and reverse primer, 5'-CCTCAGACAGACCTTTCCTC-3'. The PCR cycling for this newly isolated marker was 4
10 min. at 95°C, followed by 30 cycles of 40 s at 95°C, 2 min. at 60°C, and 1 min. at 72°C. PCR products were separated on 6% polyacrylamide gels and exposed to film.

Results and Discussion

Intronic boundaries were determined for 22 exons of *ptc* by sequencing vectorette PCR products derived from BAC 192J22 (Johnson R.L., *supra*; Table 1). Our findings are in
15 agreement with those of Hahn *et al.* (*supra*), except that we find exon 12 is composed of 2 separate exons of 126 and 119 nucleotides. This indicates that *ptc* is composed of 23 coding exons instead of 22. In addition, we find that exons 3, 4, 10, 11, 17, 21, and 23 differ slightly in size than reported previously (Hahn *et al.*, *supra*). Of 63 tumors studied, 14 were sporadic medulloblastomas, and 9 were sporadic meningiomas. These 23 tumors were examined for
20 allelic deletions by genotyping of tumor and blood DNA with microsatellite markers that flank the *ptc* gene: D9S119, D9S196, D9S287, D9S127, and D9S109. Four of 14 medulloblastomas had LOH. Two of the medulloblastomas, both of which had LOH, had mutations (med34 and med36; see Cogen, P.H. *et al.*, *supra*), which are predicted to result in truncated proteins (Table
2). DNA samples from the blood of these patients lack these mutations, indicating that they
25 both are somatic mutations. med34 also has allelic loss on 17p (Cogen, P.H. *et al.*, *supra*). We were unable to detect *ptc* gene mutations by heteroduplex analysis in the other two medulloblastomas bearing LOH on 9q. The pathological features of these two tumors differed in that med34 belongs to the desmoplastic subtype, whereas med36 is of the classic type,

5 subtypes of medulloblastomas bearing LOH on chromosome 9q22.3. Independently, another group has reported their finding of *ptc* mutations in sporadic medulloblastomas (Raffel, C. *et al, supra*).

A change of T to C at nucleotide 2990 (in exon 18) was identified in DNA from one of nine sporadic meningiomas, causing a predicted change of codon 997 from Ile to Thr (Table 2). The meningioma bearing this mutation also has allelic loss on 9q22.3. Blood cell DNA is heterozygous for this mutation, but DNA from the tumor contains only the mutant sequence. Of 100 normal chromosomes examined, none has this sequence change, suggesting that this mutation is not likely a common polymorphism. This patient is 84 years old and has had no phenotypic abnormalities suggestive of the BCNS, suggesting that this sequence alteration may not have caused complete inactivation of the *ptc* gene. None of the other eight meningiomas had detectable LOH at chromosome 9q.

TABLE 2 *PATCHED* gene alterations^a

Tumor	Pathology	Nucleotide	Codon	Exon	Consequence	LOH	Mutation Type
Med34	Medulloblastoma (desmoplastic)	TC1869A	623	14	Frameshift	Yes	Somatic
20 Med36	Medulloblastoma (classic)	G2533T	835	15	Glu to STOP	Yes	Somatic
Men1	Meningioma	T2990C	997	18	Ile to Thr	Yes	Germline
Br349	Breast carcinoma	T2843C	955	17	Tyr to His	Yes	Somatic
Br321	Breast carcinoma	A2975G	995	18	Glu to Gly	No	Somatic
Co320	Colon tumor cell line	A2000C	667	14	Glu to Ala	No	Unknown
25 Co8-1	Colon carcinoma	T to C	Intron 10		Polymorphism	No	Germline
Co15-1	Colon carcinoma	T to C	Intron 10		Polymorphism	No	Germline

We also examined a variety of other tumors (10 primary tumors and 1 cell line), 18 bladder tumors (14 primary tumors and 4 cell lines), and 2 ovarian cancer cell lines. These tumors are not known to occur in higher than expected frequency in BCNS patients. We identified sequence abnormalities in two breast carcinomas and in the one colon cancer cell line (Table 2). The mutation found in breast carcinoma Br349 is not present in the patient's normal

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5 skin DNA, indicating that the sequence change is a somatic mutation. Direct sequencing of the PCR product indicated that only the mutant allele is present in the tumor. This mutation changes codon 955 from Tyr to His, and this Tyr is conserved in human, murine, chicken, and fly *ptcII* homologues (Goodrich, L.V. *et al.* (1996) *Genes Dev* 10:301-312). The mutation in breast carcinoma Br321 is predicted to change codon 995 from Glu to Gly, and the tumor with
10 this mutation retains the wild-type allele. We have sequenced exon 18 in DNA from the blood of 50 normal persons and found no changes from the published sequence, suggesting that the sequence change found in Br321 is not a common polymorphism. Furthermore, examination of the DNA from the cultured skin fibroblasts of the patient did not reveal the same mutation, indicating that this is a somatic mutation.

15 Because DNA is not available from normal cells of the patient from which colon cell line 320 was established, we used simplified amplification of specific allele analysis (Lei, X. and Hall, B.G., *supra*) to examine 50 normal blood DNA samples for the presence of the sequence alteration and found none but the DNA from this cell line to have the mutant allele, suggesting that this mutation also is unlikely to be a common sequence polymorphism. For bladder
20 carcinomas, a newly isolated microsatellite that was derived from intron 1 of the *ptc* gene was used to examine LOH in the tumor. Three primary bladder carcinomas showed LOH at this intragenic locus. With no *ptc* mutations detected in these tumors, we suspect that the LOH in these three bladder carcinomas may reflect the high incidence of whole chromosome 9 loss in bladder cancers (Sidransky, D. *et al.*, *supra*). A similar observation has been reported
25 previously (Simoneau, A. R. *et al.* (1996) *Cancer Res* 56:5039-5043).

We also detected a sequence change in intron 10 in two colon carcinomas, 15-1 and 8-1, an alteration that was reported previously as a splicing mutation (Uden, A.B. *et al.* (1996) *Cancer Res* 56:4562-4565). Because we found the same sequence change in about 20% of

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5 normal control samples, we suggest that this more likely is a nonpathogenic polymorphism. The *ptc* protein is predicted to contain 12 transmembrane domains, two large extracellular loops, and one intracellular loop (Goodrich, L.V. *et al.*, *supra*). Of the six mutations we identified, four are missense mutations. Three mutations lead to amino acid substitutions in the second extracellular loop, and one mutation results in an amino acid change in the intracellular domain.

10 Our data indicate that somatic inactivation of the *ptc* gene does occur in some sporadic medulloblastomas. In addition, because missense mutations of the *ptc* gene were detected in breast carcinomas, we suspect that defects of the *ptc* function also may be involved in some breast carcinomas, although biochemical evidence is necessary to show how these missense mutations might impair *ptc* function. Of 11 colon cancers and 18 bladder carcinomas

15 examined, we found only one mutation in 1 colon cell line, suggesting that *ptc* gene mutations are relatively uncommon in colon and bladder cancers, although the incidence of chromosome 9 loss in bladder cancers is high (Cairns, P. *et al.*, *supra*).

Published reports of SSCP analysis of tumor DNA identified mutations in the *ptc* gene in only 30% of sporadic BCCs, although chromosome 9q22.3 LOH was reported in more than

20 50% of these tumors (Gallani, M.R. *et al.*, *supra*). It has been reported that heteroduplex/SSCP analysis of gene mutations is more sensitive than SSCP analysis (Spritz, R.A. *et al.*, *supra*). In our studies, we were able to identify a point mutation in the 310-bp PCR product from exon 15 using heteroduplex analysis, whereas SSCP analysis failed to reveal this sequence change (Table

2) . Therefore, we suspect that there may be more mutations in BCCs than we have found thus

25 far. Analysis of the *ptc* gene in BCNS patients and in sporadic BCCs has identified mutations scattered widely across the gene, and the majority of mutations were predicted to result in truncated proteins (Hahn, H. *et al.*, *supra*; Johnson, R.L. *et al.*, *supra*; Gallani, M.R. *et al.*, *supra*; Chidambaram, A. *et al.*, *supra*; Uuden, A.B. *et al.*, *supra*; Wicking, C. *et al.* (1997) *Am*

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5 *J Hum Genet* 60:21-26). In our screening, we found two breast carcinomas bearing missense mutations of the *ptc* gene. In one of these two tumors, B349, direct sequencing indicated a deletion of the other copy of the *ptc* gene. Any comparison of mutations in skin cancers versus extracutaneous tumors must consider the wholly different causes of these mutations; UV light is unique to the skin.

10 All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent o application were specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to
15 those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

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SEQUENCE LISTING

5

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20 (A) ADDRESSEE: Foley, Hoag & Eliot LLP
(B) STREET: One Post Office Square
(C) CITY: Boston
(D) STATE: MA
(E) COUNTRY: US
(F) ZIP: 02109

25 (v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

30 (vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

35 (vii) ATTORNEY/AGENT INFORMATION:

40 (A) NAME: Vincent, Matthew P.
(B) REGISTRATION NUMBER: 36,709
(C) REFERENCE/DOCKET NUMBER: SUV003.26

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 617-832-1000
(B) TELEFAX: 617-832-7000

45 (2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

50 (A) LENGTH: 736 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AACNCCNCTN NATGGCACCC CCHCCCAACC TTNNNCCNN NTAANCAAAA NNCCCNNTTT 60
NATACCCCTT NTAATGTTT TCCACCTTTC AAAAATTCN CTCATACCA NTAATTCN 120
TTTTRAAC CCCCCACCC CCAATTCNA NTNNCCNCCC CCAATTACA ACTCCAGNCC 180

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AAAATTNANA NAATTGGTCC TAACCTAACC NATNGTTGTT ACGGTTTCCC CCCCCAAATA 240
 CATGCACTGG CCCGAACACT TGATCGTTGC CGTTCCAATA AGAATAAATC TGGTCATATT 300
 AAACAAGCCN AAAGCTTTAC AAACGTGTTGT ACAATTAATG GGCGAACACG AACTGTTCGA 360
 ATTCTGGTCT GGACATTACA AAGTGCACCA CATCGGATGG AACCAGGAGA AGGCCACAAC 420
 CGTACTGAAC GCCTGGCAGA AGAAGTTCGC ACAGGTTGGT GGTGGCGCA AGGAGTAGAG 480
 TGAATGGTGG TAATTTTGG TTGTTCCAGG AGGTGGATCG TCTGACGAAG AGCAAGAAGT 540
 CGTCGAATTA CATCTTCGTG ACGTTCTCCA CCGCCAATTT GAACAAGATG TTGAAGGAGG 600
 CGTCGAANAC GGACGTGGTG AAGCTGGGGG TGGTGCTGGG GGTGGCGGCG GTGTACGGGT 660
 GGTGGCCCA GTCGGGGCTG GCTGCCTTGG GAGTGCTGGT CTTNGCGNGC TNCNATTCGC 720
 CCTATAGTNA GNCGTA 736



(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 107 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Xaa Pro Pro Pro Asn Tyr Asn Ser Xaa Pro Lys Xaa Xaa Xaa Leu Val
 1 5 10 15
 Leu Thr Pro Xaa Val Val Thr Val Ser Pro Pro Lys Tyr Met His Trp
 20 25 30
 Pro Glu His Leu Ile Val Ala Val Pro Ile Arg Ile Asn Leu Val Ile
 35 40 45
 Leu Asn Lys Pro Lys Ala Leu Gln Thr Val Val Gln Leu Met Gly Glu
 50 55 60
 His Glu Leu Phe Glu Phe Trp Ser Gly His Tyr Lys Val His His Ile
 65 70 75 80
 Gly Trp Asn Gln Glu Lys Ala Thr Thr Val Leu Asn Ala Trp Gln Lys
 85 90 95
 Lys Phe Ala Gln Val Gly Gly Trp Arg Lys Glu
 100 105

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5187 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GGGTCTGTCA CCCGGAGCCG GAGTCCCCGG CGGCCAGCAG CGTCCTCGCG AGCCGAGCGC	60
CCAGGCGCGC CCGGAGCCCC CGGCGGCGGC GGCAACATGG CCTCGGCTGG TAACGCGGCC	120
GGGGCCCTGG GCAGGCAGGC CGGCGGCGGG AGGCGCAGAC GGACCGGGGG ACCGCACCGC	180
GCCGCGCCGG ACCGGGACTA TCTGCACCGG CCCAGCTACT GCGACGCCGC CTTCGCTCTG	240
GAGCAGATT CCAAGGGGAA GGCTACTGGC CGGAAAGCGC CGCTGTGGCT GAGAGCGAAG	300
TTTCAGAGAC TCTTATTTAA ACTGGGTTGT TACATTCAA AGAACTGCGG CAAGTTTTTG	360
GTGTGGGTC TCCTCATATT TGGGGCCTTC GCTGTGGGAT TAAAGGCAGC TAATCTCGAG	420
ACCAACGTGG AGGAGCTGTG GGTGGAAGTT GGTGGACGAG TGAGTCGAGA ATTAAATTAT	480
ACCCGTCAGA AGATAGGAGA AGAGGCTATG TTTAATCCTC AACTCATGAT ACAGACTCCA	540
AAAGAAGAAG GCGCTAATGT TCTGACCACA GAGGCTCTCC TGCAACACCT GGA CTCAGCA	600
CTCCAGGCCA GTCGTGTGCA CGTCTACATG TATAACAGGC AATGGAAGTT GGAACATTG	660
TGCTACAAAT CAGGGGAACT TATCACGGAG ACAGGTTACA TGGATCAGAT AATAGAATAC	720
CTTTACCTT GCTTAATCAT TACACCTTTG GACTGCTTCT GGAAGGGGC AAAGCTACAG	780
TCCGGGACAG CATACTCCT AGGTAAGCCT CCTTTACGGT GGACAACTT TGACCCCTTG	840
GAATTCCTAG AAGAGTTAAA GAAAATAAAC TACCAAGTGG ACAGCTGGGA GGAAATGCTG	900
AATAAGCCG AAGTTGGCCA TGGGTACATG GACCGGCCTT GCCTCAACCC AGCCGACCCA	960
GATTGCCCTG CCACAGCCCC TAACAAAAAT TCAACCAAAC CTCTTGATGT GGCCCTTGTT	1020
TTGAATGGTG GATGTCAAGG TTTATCCAGG AAGTATATGC ATTGGCAGGA GGAGTTGATT	1080
GTGGGTGGTA CCGTCAAGAA TGCCACTGGA AAAC TTGTCA GCGCTCACGC CCTGCAAACC	1140
ATGTTCCAGT TAATGACTCC CAAGCAAATG TATGAACACT TCAGGGGCTA CGACTATGTC	1200
TCTCATATCA ACTGGAATGA AGACAGGGCA GCCGCCATCC TGGAGGCCTG GCAGAGGACT	1260
TACGTGGAGG TGGTTCATCA AAGTGTGCGC CCAAACCTCA CTCAAAGGT GCTTCCCTTC	1320
ACAACCACGA CCCTGGACGA CATCCTAAA TCCTTCTCTG ATGTCAGTGT CATCCGAGTG	1380
GCCAGCGGCT ACCTACTGAT GCTTGCCTAT GCCTGTTTAA CCATGCTGCG CTGGGACTGC	1440
TCCAAGTCCC AGGGTGCCGT GGGGCTGGCT GCGCTCCTGT TGGTTGCGCT GTCAGTGGCT	1500
GCAGGATTGG GCCTCTGCTC CTTGATTGGC ATTTCTTTTA ATGCTGCGAC AACTCAGGTT	1560
TTGCCGTTTC TTGCTCTTGG TGTTGGTGTG GATGATGTCT TCCTCCTGGC CCATGCATTC	1620
AGTGAACAG GACAGAATAA GAGGATTCCA TTTGAGGACA GGA CTGGGGA GTGCCCTCAAG	1680

CGCACCGGAG CCAGCGTGGC CCTCACCTCC ATCAGCAATG TCACCGCCTT CTTTCATGGCC	1740
GCATTGATCC CTATCCCTGC CCTGCGAGCG TTCTCCCTCC AGGCTGCTGT GGTGGTGGTA	1800
TTCAATTTTG CTATGGTTCT GCTCATTITT CCTGCAATTC TCAGCATGGA TTTATACAGA	1860
CGTGAGGACA GAAGATTGGA TATTTTCTGC TGTTTCACAA GCCCCTGTGT CAGCAGGGTG	1920
ATTCAAGTTG AGCCACAGGC CTACACAGAG CCTCACAGTA ACACCCGGTA CAGCCCCCA	1980
CCCCATACA CCAGCCACAG CTTGCCCCAC GAAACCCATA TCACTATGCA GTCCACCGTT	2040
CAGCTCCGCA CAGAGTATGA CCCTCACACG CACGTGTACT ACACCACCGC CGAGCCACGC	2100
TCTGAGATCT CTGTACAGCC TGTTACCGTC ACCCAGGACA ACCTCAGCTG TCAGAGTCCC	2160
GAGAGCACCA GCTCTACCAG GGACCTGCTC TCCAGTTCT CAGACTCCAG CCTCCACTGC	2220
CTCGAGCCCC CCTGCACAA GTGGACACTC TCTTCGTTT CAGAGAAGCA CTATGCTCCT	2280
TTCTCCTGA AACCCAAAGC CAAGGTTGTG GTAATCCTTC TTTTCCTGGG CTGCTGGGG	2340
GTACGCTTT ATGGGACCAC CCGAGTGAGA GACGGGCTGG ACCTCACGGA CATGTGTTCC	2400
CGGGAACCA GAGAATATGA CTTCATAGCT GCCCAGTTCA AGTACTTCTC TTTCTACAAC	2460
ATGTATATAG TCACCCAGAA AGCAGACTAC CCGAATATCC AGCACCTACT TTACGACCTT	2520
CATAAGAGTT TCAGCAATGT GAAGTATGTC ATGCTGGAGG AGAACAAGCA ACTTCCCCAA	2580
ATGTGGCTGC ACTACTTTAG AGACTGGCTT CAAGGACTTC AGGATGCATT TGACAGTGAC	2640
TGGGAAACTG GGAGGATCAT GCCAAACAAT TATAAAAATG GATCAGATGA CGGGGTCTCT	2700
GCTTACAAAC TCCTGGTGCA GACTGGCAGC CGAGACAAGC CCATCGACAT TAGTCAGTTG	2760
ACTAACAGC GTCTGGTAGA CGCAGATGGC ATCATTATC CGAGCGCTT CTACATCTAC	2820
CTGACCGCTT GGGTCAGCAA CGACCCTGTA GCTTACGCTG CCTCCCAGGC CAACATCCGG	2880
CCTCACCGGC CGGAGTGGT CCATGACAAA GCCGACTACA TGCCAGAGAC CAGGCTGAGA	2940
ATCCCAGCAG CAGAGCCCAT CGAGTACGCT CAGTTCCCTT TCTACCTCAA CGGCCTACGA	3000
GACACCTCAG ACTTTGTGGA AGCCATAGAA AAAGTGAGAG TCATCTGTAA CAACTATACG	3060
AGCCTGGGAC TGTCCAGTA CCCCAATGGC TACCCCTTCC TGTTCTGGGA GCAATACATC	3120
AGCCTGCGCC ACTGGCTGCT GCTATCCATC AGCGTGGTGC TGGCCTGCAC GTTCTAGTG	3180
TGCGCAGTCT TCCTCCTGAA CCCCTGGACG GCCGGGATCA TTGTCATGGT CCTGGCTCTG	3240
ATGACCGTTG AGCTCTTTGG CATGATGGGC CTCATTGGGA TCAAGCTGAG TGCTGTGCCT	3300
GTGGTCATCC TGATTGCATC TGTTGGCATC GGAGTGGAGT TCACCGTCCA CGTGGCTTTG	3360
GCCTTTCTGA CAGCCATTGG GGACAAGAAC CACAGGGCTA TGCTCGCTCT GGAACACATG	3420
TTTGCTCCCG TTCTGGACGG TGCTGTGTCC ACTCTGCTGG GTGTACTGAT GCTTGCAGGG	3480
TCCGAATTG ATTTCAITGT CAGATACTTC TTTGCCGTCC TGGCCATTCT CACCGTCTTG	3540
GGGGTTCTCA ATGGACTGGT TCTGCTGCCT GTCCTTTAT CCTTCTTTGG ACCGTGTCCT	3600

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GAGGTGTCTC CAGCCAATGG CCTAAACCGA CTGCCCACTC CTTCGCCTGA GCCGCCTCCA 3660
AGTGTCTGTC GGTTCGCGT GCCTCCTGGT CACACGAACA ATGGGTCTGA TTCCTCCGAC 3720
TCGGAGTACA GCTCTCAGAC CACGGTGTCT GGCATCAGTG AGGAGCTCAG GCAATACGAA 3780
GCACAGCAGG GTGCCGGAGG CCCTGCCAC CAAGTGATTG TGGAAGCCAC AGAAAACCTT 3840
GTCTTTGCCC GGTCCACTGT GGTCCATCCG GACTCCAGAC ATCAGCCTCC CTTGACCCCT 3900
CGGCAACAGC CCCACCTGGA CTCTGGCTCC TTGTCCCCTG GACGGCAAGG CCAGCAGCCT 3960
CGAAGGGATC CCCCTAGAGA AGGCTTGCGG CCACCCCTT ACAGACCGCG CAGAGACGCT 4020
TTTGAAATTT CTAAGAAGG GCATTCTGGC CTAAGAATA GGGACCGCTC AGGGCCCCGT 4080
GGGCCCCGTT CTCACAACCC TCGGAACCCA ACGTCCACCG CCATGGGCAG CTCTGTGCCC 4140
AGCTACTGCC AGCCCATCAC CACTGTGACG GCTTCTGCTT CGGTGACTGT TGCTGTGCAT 4200
CCCCCGCTG GACCTGGGCG CAACCCCGA GGGGGGCCCT GTCCAGGCTA TGAGAGCTAC 4260
CCTGAGACTG ATCAGGGGT ATTTGAGGAT CCTCATGTGC CTTTTCATGT CAGGTGTGAG 4320
AGGAGGGACT CAAAGGTGGA GGTATAGAG CTACAGGACG TGGAATGTGA GGAGAGGCCG 4380
TGGGGGAGCA GCTCCAATG AGGGTAATTA AAATCTGAAG CAAAGAGGCC AAAGATTGGA 4440
AAGCCCCGCC CCCACCTCTT TCCAGAACTG CTTGAAGAGA ACTGCTTGGA ATTATGGGAA 4500
GGCAGTTCAT TGTTACTGTA ACTGATTGTA TTATTKGTG AAATATTCT ATAAATATTT 4560
AARAGGTGTA CACATGTAAT ATACATGGAA ATGCTGTACA GTCTATTTC TGGGGCCTCT 4620
CCACTCTGC CCCAGAGTGG GGAGACCACA GGGGCCCTT CCCCTGTGTA CATTGGTCTC 4680
TGTGCCACAA CCAAGCTTAA CTTAGTTTTA AAAAAATCT CCCAGCATAT GTCGCTGCTG 4740
CTTAAATATT GTATAATTA CTTGTATAAT TCTATGCAA TATTGCTTAT GTAATAGGAT 4800
TATTTGTAAA GGTTCCTGTT TAAATATTT TAAATTTGCA TATCACAACC CTGTGGTAGG 4860
ATGAATTGTT ACTGTAACT TTTGAACAG CTATGCGTGG TAATTGTTA ACGAGCAGAC 4920
ATGAAGAAAA CAGGTTAATC CCAGTGGCTT CTCTAGGGGT AGTTGTATAT GGTTGCGATG 4980
GGTGGATGTG TGTGTGCATG TGACTTTCCA ATGACTGTA TTGTGGTTG TTGTTGTTGT 5040
TGCTGTTGTT GTTCATTTG GTGTTTTGG TTGCTTTGTA TGATCTTAGC TCTGGCCTAG 5100
GTGGGCTGGG AAGGTCCAGG TCTTTTCTG TCGTGATGCT GGTGGAAAGG TGACCCCAAT 5160
CATCTGTCCT ATTCTCTGGG ACTATTC 5187

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1311 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Val Ala Pro Asp Ser Glu Ala Pro Ser Asn Pro Arg Ile Thr Ala
1 5 10 15
Ala His Glu Ser Pro Cys Ala Thr Glu Ala Arg His Ser Ala Asp Leu
20 25 30
Tyr Ile Arg Thr Ser Trp Val Asp Ala Ala Leu Ala Leu Ser Glu Leu
35 40 45
Glu Lys Gly Asn Ile Glu Gly Gly Arg Thr Ser Leu Trp Ile Arg Ala
50 55 60
Trp Leu Gln Glu Gln Leu Phe Ile Leu Gly Cys Phe Leu Gln Gly Asp
65 70 75 80
Ala Gly Lys Val Leu Phe Val Ala Ile Leu Val Leu Ser Thr Phe Cys
85 90 95
Val Gly Leu Lys Ser Ala Gln Ile His Thr Arg Val Asp Gln Leu Trp
100 105 110
Val Gln Glu Gly Gly Arg Leu Glu Ala Glu Leu Lys Tyr Thr Ala Gln
115 120 125
Ala Leu Gly Glu Ala Asp Ser Ser Thr His Gln Leu Val Ile Gln Thr
130 135 140
Ala Lys Asp Pro Asp Val Ser Leu Leu His Pro Gly Ala Leu Leu Glu
145 150 155 160
His Leu Lys Val Val His Ala Ala Thr Arg Val Thr Val His Met Tyr
165 170 175
Asp Ile Glu Trp Arg Leu Lys Asp Leu Cys Tyr Ser Pro Ser Ile Pro
180 185 190
Asp Phe Glu Gly Tyr His His Ile Glu Ser Ile Ile Asp Asn Val Ile
195 200 205
Pro Cys Ala Ile Ile Thr Pro Leu Asp Cys Phe Trp Glu Gly Ser Lys
210 215 220
Leu Leu Gly Pro Asp Tyr Pro Ile Tyr Val Pro His Leu Lys His Lys
225 230 235 240
Leu Gln Trp Thr His Leu Asn Pro Leu Glu Val Val Glu Glu Val Lys
245 250 255
Lys Leu Lys Phe Gln Phe Pro Leu Ser Thr Ile Glu Ala Tyr Met Lys
260 265 270
Arg Ala Gly Ile Thr Ser Ala Tyr Met Lys Lys Pro Cys Leu Asp Pro
275 280 285
Thr Asp Pro His Cys Pro Ala Thr Ala Pro Asn Lys Lys Ser Gly His
290 295 300

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Ile Pro Asp Val Ala Ala Glu Leu Ser His Gly Cys Tyr Gly Phe Ala
 305 310 315 320
 Ala Ala Tyr Met His Trp Pro Glu Gln Leu Ile Val Gly Gly Ala Thr
 325 330 335
 Arg Asn Ser Thr Ser Ala Leu Arg Lys Ala Arg Xaa Leu Gln Thr Val
 340 345 350
 Val Gln Leu Met Gly Glu Arg Glu Met Tyr Glu Tyr Trp Ala Asp His
 355 360 365
 Tyr Lys Val His Gln Ile Gly Trp Asn Gln Glu Lys Ala Ala Val
 370 375 380
 Leu Asp Ala Trp Gln Arg Lys Phe Ala Ala Glu Val Arg Lys Ile Thr
 385 390 395 400
 Thr Ser Gly Ser Val Ser Ser Ala Tyr Ser Phe Tyr Pro Phe Ser Thr
 405 410 415
 Ser Thr Leu Asn Asp Ile Leu Gly Lys Phe Ser Glu Val Ser Leu Lys
 420 425 430
 Asn Ile Ile Leu Gly Tyr Met Phe Met Leu Ile Tyr Val Ala Val Thr
 435 440 445
 Leu Ile Gln Trp Arg Asp Pro Ile Arg Ser Gln Ala Gly Val Gly Ile
 450 455 460
 Ala Gly Val Leu Leu Leu Ser Ile Thr Val Ala Ala Gly Leu Gly Phe
 465 470 475 480
 Cys Ala Leu Leu Gly Ile Pro Phe Asn Ala Ser Ser Thr Gln Ile Val
 485 490 495
 Pro Phe Leu Ala Leu Gly Leu Gly Val Gln Asp Met Phe Leu Leu Thr
 500 505 510
 His Thr Tyr Val Glu Gln Ala Gly Asp Val Pro Arg Glu Glu Arg Thr
 515 520 525
 Gly Leu Val Leu Lys Lys Ser Gly Leu Ser Val Leu Leu Ala Ser Leu
 530 535 540
 Cys Asn Val Met Ala Phe Leu Ala Ala Ala Leu Leu Pro Ile Pro Ala
 545 550 555 560
 Phe Arg Val Phe Cys Leu Gln Ala Ala Ile Leu Leu Leu Phe Asn Leu
 565 570 575
 Gly Ser Ile Leu Leu Val Phe Pro Ala Met Ile Ser Leu Asp Leu Arg
 580 585 590
 Arg Arg Ser Ala Ala Arg Ala Asp Leu Leu Cys Cys Leu Met Pro Glu
 595 600 605
 Ser Pro Leu Pro Lys Lys Lys Ile Pro Glu Arg Ala Lys Thr Arg Lys
 610 615 620
 Asn Asp Lys Thr His Arg Ile Asp Thr Thr Arg Gln Pro Leu Asp Pro
 625 630 635 640

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Asp Val Ser Glu Asn Val Thr Lys Thr Cys Cys Leu Ser Val Ser Leu
 645 650 655
 Thr Lys Trp Ala Lys Asn Gln Tyr Ala Pro Phe Ile Met Arg Pro Ala
 660 665 670
 Val Lys Val Thr Ser Met Leu Ala Leu Ile Ala Val Ile Leu Thr Ser
 675 680 685
 Val Trp Gly Ala Thr Lys Val Lys Asp Gly Leu Asp Leu Thr Asp Ile
 690 695 700
 Val Pro Glu Asn Thr Asp Glu His Glu Phe Leu Ser Arg Gln Glu Lys
 705 710 715 720
 Tyr Phe Gly Phe Tyr Asn Met Tyr Ala Val Thr Gln Gly Asn Phe Glu
 725 730 735
 Tyr Pro Thr Asn Gln Lys Leu Leu Tyr Glu Tyr His Asp Gln Phe Val
 740 745 750
 Arg Ile Pro Asn Ile Ile Lys Asn Asp Asn Gly Gly Leu Thr Lys Phe
 755 760 765
 Trp Leu Ser Leu Phe Arg Asp Trp Leu Leu Asp Leu Gln Val Ala Phe
 770 775 780
 Asp Lys Glu Val Ala Ser Gly Cys Ile Thr Gln Glu Tyr Trp Cys Lys
 785 790 795 800
 Asn Ala Ser Asp Glu Gly Ile Leu Ala Tyr Lys Leu Met Val Gln Thr
 805 810 815
 Gly His Val Asp Asn Pro Ile Asp Lys Ser Leu Ile Thr Ala Gly His
 820 825 830
 Arg Leu Val Asp Lys Asp Gly Ile Ile Asn Pro Lys Ala Phe Tyr Asn
 835 840 845
 Tyr Leu Ser Ala Trp Ala Thr Asn Asp Ala Leu Ala Tyr Gly Ala Ser
 850 855 860
 Gln Gly Asn Leu Lys Pro Gln Pro Gln Arg Trp Ile His Ser Pro Glu
 865 870 875 880
 Asp Val His Leu Glu Ile Lys Lys Ser Ser Pro Leu Ile Tyr Thr Gln
 885 890 895
 Leu Pro Phe Tyr Leu Ser Gly Leu Ser Asp Thr Xaa Ser Ile Lys Thr
 900 905 910
 Leu Ile Arg Ser Val Arg Asp Leu Cys Leu Lys Tyr Glu Ala Lys Gly
 915 920 925
 Leu Pro Asn Phe Pro Ser Gly Ile Pro Phe Leu Phe Trp Glu Gln Tyr
 930 935 940
 Leu Tyr Leu Arg Thr Ser Leu Leu Leu Ala Leu Ala Cys Ala Leu Ala
 945 950 955 960
 Ala Val Phe Ile Ala Val Met Val Leu Leu Leu Asn Ala Trp Ala Ala
 965 970 975

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Val Leu Val Thr Leu Ala Leu Ala Thr Leu Val Leu Gln Leu Leu Gly
 980 985 990
 Val Met Ala Leu Leu Gly Val Lys Leu Ser Ala Met Pro Ala Val Leu
 995 1000 1005
 Leu Val Leu Ala Ile Gly Arg Gly Val His Phe Thr Val His Leu Cys
 1010 1015 1020
 Leu Gly Phe Val Thr Ser Ile Gly Cys Lys Arg Arg Arg Ala Ser Leu
 1025 1030 1035 1040
 Ala Leu Glu Ser Val Leu Ala Pro Val Val His Gly Ala Leu Ala Ala
 1045 1050 1055
 Ala Leu Ala Ala Ser Met Leu Ala Ala Ser Glu Cys Gly Phe Val Ala
 1060 1065 1070
 Arg Leu Phe Leu Arg Leu Leu Leu Asp Ile Val Phe Leu Gly Leu Ile
 1075 1080 1085
 Asp Gly Leu Leu Phe Phe Pro Ile Val Leu Ser Ile Leu Gly Pro Ala
 1090 1095 1100
 Ala Glu Val Arg Pro Ile Glu His Pro Glu Arg Leu Ser Thr Pro Ser
 1105 1110 1115 1120
 Pro Lys Cys Ser Pro Ile His Pro Arg Lys Ser Ser Ser Ser Ser Gly
 1125 1130 1135
 Gly Gly Asp Lys Ser Ser Arg Thr Ser Lys Ser Ala Pro Arg Pro Cys
 1140 1145 1150
 Ala Pro Ser Leu Thr Thr Ile Thr Glu Glu Pro Ser Ser Trp His Ser
 1155 1160 1165
 Ser Ala His Ser Val Gln Ser Ser Met Gln Ser Ile Val Val Gln Pro
 1170 1175 1180
 Glu Val Val Val Glu Thr Thr Thr Tyr Asn Gly Ser Asp Ser Ala Ser
 1185 1190 1195 1200
 Gly Arg Ser Thr Pro Thr Lys Ser Ser His Gly Gly Ala Ile Thr Thr
 1205 1210 1215
 Thr Lys Val Thr Ala Thr Ala Asn Ile Lys Val Glu Val Val Thr Pro
 1220 1225 1230
 Ser Asp Arg Lys Ser Arg Arg Ser Tyr His Tyr Tyr Asp Arg Arg Arg
 1235 1240 1245
 Asp Arg Asp Glu Asp Arg Asp Arg Asp Arg Glu Arg Asp Arg Asp Arg
 1250 1255 1260
 Asp Arg Asp Arg Asp Arg Asp Arg Asp Arg Asp Arg Asp Arg Asp Arg
 1265 1270 1275 1280
 Glu Arg Ser Arg Glu Arg Asp Arg Arg Asp Arg Tyr Arg Asp Glu Arg
 1285 1290 1295
 Asp His Arg Ala Ser Pro Arg Glu Lys Arg Gln Arg Phe Trp Thr
 1300 1305 1310

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4434 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CGAAACAAGA CAGCGAGTGA GAGTAGGGAG AGCGTCTGTG TTGTGTGTTG AGTGTGCCCC	60
ACGCACACAG GCGCAAAACA GTGCACACAG ACGCCCGCTG GGCAAGAGAG AGTGAGAGAG	120
AGAAACAGCG GCGCGCGCTC GCCTAATGAA GTTGTGTGGC TGGCTGGCGT GCCGCATCCA	180
CGAGATACAG ATACATCTCT CATGGACCGC GACAGCCTCC CACGCGTTCC GGACACACAC	240
GGCGATGTGG TCGATGAGAA ATTATTCTCG GATCTTTACA TACGCACCAG CTGGGTGGAC	300
GCGCAAGTGG CGCTCGATCA GATAGATAAG GGCAAAGCGC GTGGCAGCCG CACGGCGATC	360
TATCTGCGAT CAGTATTCCA GTCCACCTC GAAACCCTCG GCAGCTCCGT GCAAAAGCAC	420
GCGGGCAAGG TGCTATTTCG GGCTATCCTG GTGCTGAGCA CCTTCTGCGT CGGCCTGAAG	480
AGCGCCCAGA TCCACTCCAA GGTGCACCAG CTGTGGATCC AGGAGGGCGG CCGGCTGGAG	540
GCGGAACTGG CCTACACACA GAAGACGATC GCGGAGGACG AGTCGGCCAC GCATCAGCTG	600
CTCATTCAGA CGACCCACGA CCCGAACGCC TCCGTCCTGC ATCCGCAGGC GCTGCTTGCC	660
CACCTGGAGG TCCTGGTCAA GGCCACCGCC GTCAAGGTGC ACCTCTACGA CACCGAATGG	720
GGGTGCGCG ACATGTGCAA CATGCCGAGC ACGCCCTCCT TCGAGGGCAT CTACTACATC	780
GAGCAGATCC TGCGCCACCT CATTCCTGTC TCGATCATCA CGCCGCTGGA CTGTTTCTGG	840
GAGGGAAGCC AGCTGTTGGG TCCGGAATCA GCGGTCGTTA TACCAGGCCT CAACCAACGA	900
CTCCTGTGGA CCACCCTGAA TCCCGCCTCT GTGATGCAGT ATATGAAACA AAAGATGTCC	960
GAGGAAAAGA TCAGCTTCGA CTTCGAGACC GTGGAGCAGT ACATGAAGCG TGCGGCCATT	1020
GGCAGTGGCT ACATGGAGAA GCCCTGCCTG AACCCTACTGA ATCCCAATTG CCCGGACACG	1080
GCACCGAACA AGAACAGCAC CCAGCCGCCG GATGTGGGAG CCATCCTGTC CGGAGGCTGC	1140
TACGGTTATG CCGCGAAGCA CATGCACTGG CCGGAGGAGC TGATTGTGGG CGGACGGAAG	1200
AGGAACCGCA GCGGACACTT GAGGAAGGCC CAGGCCCTGC AGTCGGTGGT GCAGCTGATG	1260
ACCGAGAAGG AAATGTACGA CCAGTGGCAG GACAACATA AGGTGCACCA TCTTGGATGG	1320
ACGCAGGAGA AGGCAGCGGA GGTTTGAAC GCCTGGCAGC GCAACTTTTC GCGGGAGGTG	1380
GAACAGCTGC TACGTAAACA GTCGAGAATT GCCACCAACT ACGATATCTA CGTGTTACG	1440

TCGGCTGCAC TGGATGACAT CCTGGCCAAG TTCTCCCATC CCAGCGCCTT GTCCATTGTC 1500
ATCGGCGTGG CCGTCACCGT TTTGTATGCC TTTTGACAGC TCCTCCGCTG GAGGGACCCC 1560
GTCCGTGGCC AGAGCAGTGT GGGCGTGGCC GGAGTTCTGC TCATGTGCTT CAGTACCGCC 1620
GCGGATTGG GATTGTCAGC CCTGCTCGGT ATCGTTTTCA ATGCGCTGAC CGCTGCCTAT 1680
GCGGAGAGCA ATCGGCGGGA GCAGACCAAG CTGATTCTCA AGAACGCCAG CACCCAGGTG 1740
GTTCGTTTT TGGCCCTTGG TCTGGGCGTC GATCACATCT TCATAGTGGG ACCGAGCATC 1800
CTGTTCACTG CCTGCAGCAC CGCAGGATCC TTCTTTGCGG CCGCCTTAT TCCGGTGCCG 1860
GCTTTGAAGG TATTCTGTCT GCAGGCTGCC ATCGTAATGT GCTCCAATT GGCAGCGGCT 1920
CTATTGGTTT TTCCGGCCAT GATTTCGTTG GATCTACGGA GACGTACCGC CGGCAGGGCG 1980
GACATCTTCT GCTGCTGTTT TCCGGTGTGG AAGGAACAGC CGAAGGTGGC ACCTCCGGTG 2040
CTGCCGCTGA ACAACAACAA CGGGCGCGGG GCCCGGCATC CGAAGAGCTG CAACAACAAC 2100
AGGCTGCCGC TGCCCGCCCA GAATCCTCTG CTGGAACAGA GGGCAGACAT CCCTGGGAGC 2160
AGTCACTCAC TGGCGTCCCT CTCCTGGCA ACCTTCGCCT TTCAGCACTA CACTCCCTTC 2220
CTCATGCGCA GCTGGGTGAA GTTCCTGACC GTTATGGGT TCCTGGCGGC CCTCATATCC 2280
AGCTTGATG CCTCCACGCG CCTTCAGGAT GGCCTGGACA TTATTGATCT GGTGCCCAAG 2340
GACAGCAACG AGCACAAGT CCTGGATGCT CAAACTCGGC TCTTTGGCTT CTACAGCATG 2400
TATGCGGTTA CCCAGGGCAA CTTTGAATAT CCCACCCAGC AGCAGTTGCT CAGGGACTAC 2460
CATGATTCCT TTGTGCGGT GCCACATGTG ATCAAGAATG ATAACGGTGG ACTGCCGGAC 2520
TTCTGGCTGC TGCTCTTCAG CGAGTGGCTG GGTAATCTGC AAAAGATATT CGACGAGGAA 2580
TACCGCGACG GACGGCTGAC CAAGGAGTGC TGGTTCCCA ACGCCAGCAG CGATGCCATC 2640
CTGGCCTACA AGCTAATCGT GCAAACCGGC CATGTGGACA ACCCGTGGG CAAGGAACTG 2700
GTGCTACCA ATCGCCTGGT CAACAGCGAT GGCATCATCA ACCAACGCGC CTTCTACAAC 2760
TATCTGTGCG CATGGGCCAC CAACGACGTC TTCGCCTACG GAGCTTCTCA GGGCAAATTG 2820
TATCCGGAAC CGCGCCAGTA TTTTACCAA CCCAACGAGT ACGATCTTAA GATACCCAAG 2880
AGTCTGCCAT TGGTCTACGC TCAGATGCC TTTTACCTCC ACGGACTAAC AGATACCTCG 2940
CAGATCAAGA CCCTGATAGG TCATATTCGC GACCTGAGCG TCAAGTACGA GGGCTTCGGC 3000
CTGCCCAACT ATCCATCGGG CATTCCCTTC ATCTTCTGGG AGCAGTACAT GACCCTGCGC 3060
TCCTCACTGG CCATGATCCT GGCCTGCGTG CTACTCGCG CCCTGGTGCT GGTCTCCCTG 3120
CTCCTGCTCT CCGTTTGGGC CGCCGTTCTC GTGATCCTCA GCGTTCTGGC CTCGCTGGCC 3180
CAGATCTTTG GGGCCATGAC TCTGCTGGG ATCAAACTCT CGGCCATTCC GGCAGTCATA 3240
CTCATCCTCA GCGTGGGCAT GATGCTGTGC TTCAATGTGC TGATATCACT GGGCTTCATG 3300
ACATCCGTTG GCAACCGACA GCGCCGCGTC CAGCTGAGCA TGCAGATGTC CCTGGGACCA 3360

CTTGTCACG GCATGCTGAC CTCCGGAGTG GCCGTGTTCA TGCTCTCCAC GTCGCCCTTT 3420
 GAGTTTGTGA TCCGGCACTT CTGCTGGCTT CTGCTGGTGG TCTTATGCGT TGGCGCCTGC 3480
 AACAGCCTTT TGGTGTTCCT CATCCTACTG AGCATGGTGG GACCGGAGGC GGAGCTGGTG 3540
 CCGCTGGAGC ATCCAGACCG CATATCCACG CCCTCTCCGC TGCCCGTGCG CAGCAGCAAG 3600
 AGATCGGGCA AATCCTATGT GGTGCAGGGA TCGCGATCCT CGCGAGGCAG CTGCCAGAAG 3660
 TCGCATCACC ACCACCACAA AGACCTTAAT GATCCATCGC TGACGACGAT CACCGAGGAG 3720
 CCGCAGTCGT GGAAGTCCAG CAACTCGTCC ATCCAGATGC CCAATGATTG GACCTACCAG 3780
 CCGCGGGAAC AGCGACCCGC CTCCTACGCG GCGCCGCCCC CCGCCTATCA CAAGGCCGCC 3840
 GCGCAGCAGC ACCACCAGCA TCAGGGCCCG CCCACAACGC CCGCGCCTCC CTTCCCGACG 3900
 GCCTATCCGC CGGAGCTGCA GAGCATCGTG GTGCAGCCGG AGGTGACGGT GGAGACGACG 3960
 CACTCGGACA GCAACACCAC CAAGGTGACG GCCACGGCCA ACATCAAGGT GGAGCTGGCC 4020
 ATGCCCCGCA GGGCGGTGCG CAGCTATAAC TTTACGAGTT AGCACTAGCA CTAGTTCCTG 4080
 TAGCTATTAG GACGTATCTT TAGACTCTAG CCTAAGCCGT AACCTATTT GTATCTGTAA 4140
 AATCGATTTG TCCAGCGGGT CTGCTGAGGA TTTCGTTCTC ATGGATTCTC ATGGATTCTC 4200
 ATGGATGCTT AAATGGCATG GTAATTGGCA AAATATCAAT TTTTGTGTCT CAAAAAGATG 4260
 CATTAGCTTA TGGTTTCAAG ATACATTTT AAAGAGTCCG CCAGATATTT ATATAAAAAA 4320
 AATCCAAAAT CGACGTATCC ATGAAAATTG AAAAGCTAAG CAGACCCGTA TGTATGTATA 4380
 TGTGTATGCA TGTTAGTTAA TTCCCGAAG TCCGGTATTT ATAGCAGCTG CCTT 4434

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1285 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Asp Arg Asp Ser Leu Pro Arg Val Pro Asp Thr His Gly Asp Val
 1 5 10 15
 Val Asp Glu Lys Leu Phe Ser Asp Leu Tyr Ile Arg Thr Ser Trp Val
 20 25 30
 Asp Ala Gln Val Ala Leu Asp Gln Ile Asp Lys Gly Lys Ala Arg Gly
 35 40 45
 Ser Arg Thr Ala Ile Tyr Leu Arg Ser Val Phe Gln Ser His Leu Glu
 50 55 60

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Thr Leu Gly Ser Ser Val Gln Lys His Ala Gly Lys Val Leu Phe Val
 65 70 75 80
 Ala Ile Leu Val Leu Ser Thr Phe Cys Val Gly Leu Lys Ser Ala Gln
 85 90 95
 Ile His Ser Lys Val His Gln Leu Trp Ile Gln Glu Gly Gly Arg Leu
 100 105 110
 Glu Ala Glu Leu Ala Tyr Thr Gln Lys Thr Ile Gly Glu Asp Glu Ser
 115 120 125
 Ala Thr His Gln Leu Leu Ile Gln Thr Thr His Asp Pro Asn Ala Ser
 130 135 140
 Val Leu His Pro Gln Ala Leu Leu Ala His Leu Glu Val Leu Val Lys
 145 150 155 160
 Ala Thr Ala Val Lys Val His Leu Tyr Asp Thr Glu Trp Gly Leu Arg
 165 170 175
 Asp Met Cys Asn Met Pro Ser Thr Pro Ser Phe Glu Gly Ile Tyr Tyr
 180 185 190
 Ile Glu Gln Ile Leu Arg His Leu Ile Pro Cys Ser Ile Ile Thr Pro
 195 200 205
 Leu Asp Cys Phe Trp Glu Gly Ser Gln Leu Leu Gly Pro Glu Ser Ala
 210 215 220
 Val Val Ile Pro Gly Leu Asn Gln Arg Leu Leu Trp Thr Thr Leu Asn
 225 230 235 240
 Pro Ala Ser Val Met Gln Tyr Met Lys Gln Lys Met Ser Glu Glu Lys
 245 250 255
 Ile Ser Phe Asp Phe Glu Thr Val Glu Gln Tyr Met Lys Arg Ala Ala
 260 265 270
 Ile Gly Ser Gly Tyr Met Glu Lys Pro Cys Leu Asn Pro Leu Asn Pro
 275 280 285
 Asn Cys Pro Asp Thr Ala Pro Asn Lys Asn Ser Thr Gln Pro Pro Asp
 290 295 300
 Val Gly Ala Ile Leu Ser Gly Gly Cys Tyr Gly Tyr Ala Ala Lys His
 305 310 315 320
 Met His Trp Pro Glu Glu Leu Ile Val Gly Gly Arg Lys Arg Asn Arg
 325 330 335
 Ser Gly His Leu Arg Lys Ala Gln Ala Leu Gln Ser Val Val Gln Leu
 340 345 350
 Met Thr Glu Lys Glu Met Tyr Asp Gln Trp Gln Asp Asn Tyr Lys Val
 355 360 365
 His His Leu Gly Trp Thr Gln Glu Lys Ala Ala Glu Val Leu Asn Ala
 370 375 380
 Trp Gln Arg Asn Phe Ser Arg Glu Val Glu Gln Leu Leu Arg Lys Gln
 385 390 395 400

60

Ser Arg Ile Ala Thr Asn Tyr Asp Ile Tyr Val Phe Ser Ser Ala Ala
 405 410 415
 Leu Asp Asp Ile Leu Ala Lys Phe Ser His Pro Ser Ala Leu Ser Ile
 420 425 430
 Val Ile Gly Val Ala Val Thr Val Leu Tyr Ala Phe Cys Thr Leu Leu
 435 440 445
 Arg Trp Arg Asp Pro Val Arg Gly Gln Ser Ser Val Gly Val Ala Gly
 450 455 460
 Val Leu Leu Met Cys Phe Ser Thr Ala Ala Gly Leu Gly Leu Ser Ala
 465 470 475 480
 Leu Leu Gly Ile Val Phe Asn Ala Leu Thr Ala Ala Tyr Ala Glu Ser
 485 490 495
 Asn Arg Arg Glu Gln Thr Lys Leu Ile Leu Lys Asn Ala Ser Thr Gln
 500 505 510
 Val Val Pro Phe Leu Ala Leu Gly Leu Gly Val Asp His Ile Phe Ile
 515 520 525
 Val Gly Pro Ser Ile Leu Phe Ser Ala Cys Ser Thr Ala Gly Ser Phe
 530 535 540
 Phe Ala Ala Ala Phe Ile Pro Val Pro Ala Leu Lys Val Phe Cys Leu
 545 550 555 560
 Gln Ala Ala Ile Val Met Cys Ser Asn Leu Ala Ala Ala Leu Leu Val
 565 570 575
 Phe Pro Ala Met Ile Ser Leu Asp Leu Arg Arg Arg Thr Ala Gly Arg
 580 585 590
 Ala Asp Ile Phe Cys Cys Cys Phe Pro Val Trp Lys Glu Gln Pro Lys
 595 600 605
 Val Ala Pro Pro Val Leu Pro Leu Asn Asn Asn Asn Gly Arg Gly Ala
 610 615 620
 Arg His Pro Lys Ser Cys Asn Asn Asn Arg Val Pro Leu Pro Ala Gln
 625 630 635 640
 Asn Pro Leu Leu Glu Gln Arg Ala Asp Ile Pro Gly Ser Ser His Ser
 645 650 655
 Leu Ala Ser Phe Ser Leu Ala Thr Phe Ala Phe Gln His Tyr Thr Pro
 660 665 670
 Phe Leu Met Arg Ser Trp Val Lys Phe Leu Thr Val Met Gly Phe Leu
 675 680 685
 Ala Ala Leu Ile Ser Ser Leu Tyr Ala Ser Thr Arg Leu Gln Asp Gly
 690 695 700
 Leu Asp Ile Ile Asp Leu Val Pro Lys Asp Ser Asn Glu His Lys Phe
 705 710 715 720
 Leu Asp Ala Gln Thr Arg Leu Phe Gly Phe Tyr Ser Met Tyr Ala Val
 725 730 735
 Thr Gln Gly Asn Phe Glu Tyr Pro Thr Gln Gln Gln Leu Leu Arg Asp

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740	745	750
Tyr His Asp Ser Phe Arg Val	Pro His Val Ile Lys Asn Asp Asn Gly	
755	760	765
Gly Leu Pro Asp Phe Trp Leu	Leu Leu Phe Ser Glu Trp Leu Gly Asn	
770	775	780
Leu Gln Lys Ile Phe Asp Glu	Glu Tyr Arg Asp Gly Arg Leu Thr Lys	
785	790	800
Glu Cys Trp Phe Pro Asn Ala	Ser Ser Asp Ala Ile Leu Ala Tyr Lys	
805	810	815
Leu Ile Val Gln Thr Gly His	Val Asp Asn Pro Val Asp Lys Glu Leu	
820	825	830
Val Leu Thr Asn Arg Leu Val	Asn Ser Asp Gly Ile Ile Asn Gln Arg	
835	840	845
Ala Phe Tyr Asn Tyr Leu Ser	Ala Trp Ala Thr Asn Asp Val Phe Ala	
850	855	860
Tyr Gly Ala Ser Gln Gly Lys	Leu Tyr Pro Glu Pro Arg Gln Tyr Phe	
865	870	875
His Gln Pro Asn Glu Tyr Asp	Leu Lys Ile Pro Lys Ser Leu Pro Leu	
885	890	895
Val Tyr Ala Gln Met Pro Phe	Tyr Leu His Gly Leu Thr Asp Thr Ser	
900	905	910
Gln Ile Lys Thr Leu Ile Gly	His Ile Arg Asp Leu Ser Val Lys Tyr	
915	920	925
Glu Gly Phe Gly Leu Pro Asn	Tyr Pro Ser Gly Ile Pro Phe Ile Phe	
930	935	940
Trp Glu Gln Tyr Met Thr Leu	Arg Ser Ser Leu Ala Met Ile Leu Ala	
945	950	955
Cys Val Leu Leu Ala Ala Leu	Val Leu Val Ser Leu Leu Leu Leu Ser	
965	970	975
Val Trp Ala Ala Val Leu Val	Ile Leu Ser Val Leu Ala Ser Leu Ala	
980	985	990
Gln Ile Phe Gly Ala Met Thr	Leu Leu Gly Ile Lys Leu Ser Ala Ile	
995	1000	1005
Pro Ala Val Ile Leu Ile Leu	Ser Val Gly Met Met Leu Cys Phe Asn	
1010	1015	1020
Val Leu Ile Ser Leu Gly Phe	Met Thr Ser Val Gly Asn Arg Gln Arg	
1025	1030	1035
Arg Val Gln Leu Ser Met Gln	Met Ser Leu Gly Pro Leu Val His Gly	
1045	1050	1055
Met Leu Thr Ser Gly Val Ala	Val Phe Met Leu Ser Thr Ser Pro Phe	
1060	1065	1070
Glu Phe Val Ile Arg His Phe	Cys Trp Leu Leu Val Val Leu Cys	

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1075	1080	1085
Val Gly Ala Cys Asn Ser Leu Leu Val Phe Pro Ile Leu Leu Ser Met 1090	1095	1100
Val Gly Pro Glu Ala Glu Leu Val Pro Leu Glu His Pro Asp Arg Ile 1105	1110	1115 1120
Ser Thr Pro Ser Pro Leu Pro Val Arg Ser Ser Lys Arg Ser Gly Lys 1125	1130	1135
Ser Tyr Val Val Gln Gly Ser Arg Ser Ser Arg Gly Ser Cys Gln Lys 1140	1145	1150
Ser His His His His His Lys Asp Leu Asn Asp Pro Ser Leu Thr Thr 1155	1160	1165
Ile Thr Glu Glu Pro Gln Ser Trp Lys Ser Ser Asn Ser Ser Ile Gln 1170	1175	1180
Met Pro Asn Asp Trp Thr Tyr Gln Pro Arg Glu Gln Arg Pro Ala Ser 1185	1190	1195 1200
Tyr Ala Ala Pro Pro Pro Ala Tyr His Lys Ala Ala Ala Gln Gln His 1205	1210	1215
His Gln His Gln Gly Pro Pro Thr Thr Pro Pro Pro Pro Phe Pro Thr 1220	1225	1230
Ala Tyr Pro Pro Glu Leu Gln Ser Ile Val Val Gln Pro Glu Val Thr 1235	1240	1245
Val Glu Thr Thr His Ser Asp Ser Asn Thr Thr Lys Val Thr Ala Thr 1250	1255	1260
Ala Asn Ile Lys Val Glu Leu Ala Met Pro Gly Arg Ala Val Arg Ser 1265	1270	1275 1280
Tyr Asn Phe Thr Ser 1285		

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 345 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

AAGGTCCATC AGCTTTGGAT ACAGGAAGGT GGTTCGCTCG AGCATGAGCT AGCCTACACG	60
CAGAAATCGC TCGGCGAGAT GGA CTCTCC ACGCACCAGC TGCTAATCCA AACNCCCAAA	120
GATATGGACG CCTCGATACT GCACCCGAAC GCGCTACTGA CGCACCTGGA CGTGGTGAAG	180
AAAGCGATCT CGGTGACGGT GCACATGTAC GACATCACGT GGAGNCTCAA GGACATGTGC	240

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TACTCGCCCA GCATACCGAG NTTCGATACG CACTTTATCG AGCAGATCTT CGAGAACATC 300

ATACCGTGCG CGATCATCAC GCCGCTGGAT TGCTTTTGGG AGGGA 345

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 115 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Lys Val His Gln Leu Trp Ile Gln Glu Gly Gly Ser Leu Glu His Glu
 1 5 10 15
 Leu Ala Tyr Thr Gln Lys Ser Leu Gly Glu Met Asp Ser Ser Thr His
 20 25 30
 Gln Leu Leu Ile Gln Thr Pro Lys Asp Met Asp Ala Ser Ile Leu His
 35 40 45
 Pro Asn Ala Leu Leu Thr His Leu Asp Val Val Lys Lys Ala Ile Ser
 50 55 60
 Val Thr Val His Met Tyr Asp Ile Thr Trp Xaa Leu Lys Asp Met Cys
 65 70 75 80
 Tyr Ser Pro Ser Ile Pro Xaa Phe Asp Thr His Phe Ile Glu Gln Ile
 85 90 95
 Phe Glu Asn Ile Ile Pro Cys Ala Ile Ile Thr Pro Leu Asp Cys Phe
 100 105 110
 Trp Glu Gly
 115

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5187 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GGGTCTGTCA CCCGAGCCG GASTCCCCGG CGGCCAGCAG CGTCCTCGCG AGCCGAGCGC 60
 CCAAGCGCGCG CCGGAGCCCG CGGCGGCGGC GGCAACATGG CCTCGGCTGG TAACGCCGCC 120

GGGGCCCTGG GCAGGCAGGC CGGCGGCGGG AGGCGCAGAC GGACCGGGGG ACCGCACCGC 180
GCCGCGCCGG ACCGGGACTA TCTGCACCGG CCCAGCTACT GCGACGCCGC CTTCGCTCTG 240
GAGCAGATTT CCAAGGGGAA GGCTACTGGC CGGAAAGCGC CGCTGTGGCT GAGAGCGAAG 300
TTTCAGAGAC TCTTATTTAA ACTGGGTGTG TACATTCAA AGAACTGCGG CAAGTTTTTG 360
GTGTGGGTC TCCTCATATT TGGGGCCTTC GCTGTGGGAT TAAAGGCAGC TAATCTCGAG 420
ACCAACGTGG AGGAGCTGTG GGTGGAAGTT GGTGGACGAG TGAGTCGAGA ATTAAATTAT 480
ACCCGTCAGA AGATAGGAGA AGAGGCTATG TTTAATCCTC AACTCATGAT ACAGACTCCA 540
AAAGAAGAAG GCGCTAATGT TCTGACCACA GAGGCTCTCC TGCAACACCT GGAATCAGCA 600
CTCCAGGCCA GTCGTGTGCA CGTCTACATG TATAACAGGC AATGGAAGTT GGAACATTTG 660
TGCTACAAAT CAGGGGAACT TATCACGGAG ACAGGTTACA TGGATCAGAT AATAGAATAC 720
CTTTACCCIT GCTTAATCAT TACACCTTG GACTGCTTCT GGAAGGGGC AAAGCTACAG 780
TGGGGACAG CATACCTCCT AGGTAAGCCT CCTTTACGGT GGACAAACTT TGACCCCTTG 840
GAATTCCTAG AAGAGTTAAA GAAAATAAAC TACCAAGTGG ACAGCTGGGA GGAATGCTG 900
AATAAAGCCG AAGTTGGCCA TGGGTACATG GACCGGCCTT GCCTCAACCC AGCCGACCCA 960
GATTGCCCTG CCACAGCCCC TAACAAAAAT TCAACCAAAC CTCTTGATGT GGCCCTTGTT 1020
TTGAATGGTG GATGTCAAGG TTTATCCAGG AAGTATATGC ATTGGCAGGA GGAGTTGATT 1080
GTGGGTGGTA CCGTCAAGAA TGCCACTGGA AACTTGTC GCGCTCACGC CCTGCAAACC 1140
ATGTTCCAGT TAATGACTCC CAAGCAAATG TATGAACACT TCAGGGGCTA CGACTATGTC 1200
TCTCATATCA ACTGGAATGA AGACAGGGCA GCCGCCATCC TGGAGGCCTG GCAGAGGACT 1260
TACGTGGAGG TGGTTCATCA AAGTGTGCGC CCAAACCTCCA CTCAAAGGT GCTTCCCTTC 1320
ACAACCACGA CCCTGGACGA CATCCTAAAA TCCTTCTCTG ATGTCAGTGT CATCCGAGTG 1380
GCCAGCGGCT ACCTACTGAT GCTTGCCTAT GCCTGTTTAA CCATGCTGCG CTGGGACTGC 1440
TCCAAGTCCC AGGGTGCCGT GGGGCTGGCT GCGCTCCTGT TGGTTGCGCT GTCAGTGGCT 1500
GCAGGATTGG GCCTCTGCTC CTTGATTGGC ATTTCTTTTA ATGCTGCGAC AACTCAGGTT 1560
TTGCCGTTTC TTGCTCTTGG TGTTGGTGTG GATGATGTCT TCCTCCTGGC CCATGCATTC 1620
AGTGAAACAG GACAGAATAA GAGGATTCCA TTTGAGGACA GGAATGGGA GTGCCTCAAG 1680
CGCACCGGAG CCAGCGTGGC CCTCACCTCC ATCAGCAATG TCACCGCCTT CTTTCATGGC 1740
GCATTGATCC CTATCCCTGC CCTGCGAGCG TTCTCCCTCC AGGCTGCTGT GGTGGTGGTA 1800
TTCAATTTTG CTATGGTTCT GCTCATTTTT CCTGCAATTC TCAGCATGGA TTTATACAGA 1860
CGTGAGGACA GAAGATTGGA TATTTCTGTC TGTTTCACAA GCCCCTGTGT CAGCAGGGTG 1920
ATTCAAGTTG AGCCACAGGC CTACACAGAG CCTCACAGTA ACACCCGGTA CAGCCCCCA 1980
CCCCATACA CCAGCCACAG CTTGCCCCAC GAAACCCATA TCACTATGCA GTCCACCGTT 2040

CAGCTCCGCA CAGAGTATGA CCCTCACAG CACGTGTACT ACACCACCGC CGAGCCACGC 2100
TCTGAGATCT CTGTACAGCC TGTACCGTC ACCCAGGACA ACCTCAGCTG TCAGAGTCCC 2160
GAGAGCACCA GCTCTACCAG GGACCTGCTC TCCCAGTTCT CAGACTCCAG CCTCCACTGC 2220
CTCGAGCCCC CCTGCACCAA GTGGACACTC TCTTCGTTTG CAGAGAAGCA CTATGCTCCT 2280
TTCTCTCTGA AACCCAAAGC CAAGGTGTG GTAATCCTTC TTTTCCTGGG CTTGCTGGGG 2340
GTCAGCCTTT ATGGGACCAC CCGAGTGAGA GACGGGCTGG ACCTCACGGA CATTGTTCCC 2400
CGGGAACCA GAGAATATGA CTTATAGCT GCCCAGTTCA AGTACTTCTC TTTCTACAAC 2460
ATGTATATAG TCACCCAGAA AGCAGACTAC CCGAATATCC AGCACCTACT TTACGACCTT 2520
CATAAGAGTT TCAGCAATGT GAAGTATGTC ATGCTGGAGG AGAACAAGCA ACTTCCCCAA 2580
ATGTGGCTGC ACTACTTTAG AGACTGGCTT CAAGGACTTC AGGATGCATT TGACAGTGAC 2640
TGGGAAACTG GGAGGATCAT GCCAAACAAT TATAAAATG GATCAGATGA CGGGGTCCTC 2700
GCTTACAAAC TCCTGGTGCA GACTGGCAGC CGAGACAAGC CCATCGACAT TAGTCAGTTG 2760
ACTAACAGC GTCTGGTAGA CGCAGATGGC ATCATTAAATC CGAGCGCTTT CTACATCTAC 2820
CTGACCGCTT GGGTCAGCAA CGACCCTGTA GCTTACGCTG CCTCCCAGGC CAACATCCGG 2880
CCTCACCGGC CGGAGTGGGT CCATGACAAA GCCGACTACA TGCCAGAGAC CAGGCTGAGA 2940
ATCCCAGCAG CAGAGCCCAT CGAGTACGCT CAGTTCCTT TCTACCTCAA CGGCCTACGA 3000
GACACCTCAG ACTTTGTGGA AGCCATAGAA AAAGTGAGAG TCATCTGTAA CAACTATACG 3060
AGCCTGGGAC TGTCAGCTA CCCCATGGC TACCCCTTCC TGTTCTGGGA GCAATACATC 3120
AGCCTGCGCC ACTGGCTGCT GCTATCCATC AGCGTGGTGC TGGCCTGCAC GTTCTAGTG 3180
TGCGCAGTCT TCCTCTGAA CCCCTGGACG GCCGGGATCA TTGTCATGGT CCTGGCTCTG 3240
ATGACCGTTG AGCTCTTTGG CATGATGGGC CTCATTGGGA TCAAGCTGAG TGCTGTGCCT 3300
GTGGTCAATC TGATTGCATC TGTTGGCATC GGAGTGGAGT TCACCGTCCA CGTGGCTTTG 3360
GCCTTTCTGA CAGCCATTGG GGACAAGAAC CACAGGGCTA TGCTCGCTCT GGAACACATG 3420
TTTGCTCCCG TTCTGGACGG TGCTGTGTCC ACTCTGCTGG GTGTACTGAT GCTTGACGGG 3480
TCCGAATTTG ATTTCAATGT CAGATACTTC TTTGCCGTCC TGGCCATTCT CACCGTCTTG 3540
GGGGTICTCA ATGGACTGGT TCTGCTGCCT GTCCTCTTAT CCTTCTTTGG ACCGTGTCTT 3600
GAGGTGTCTC CAGCCAATGG CCTAAACCGA CTGCCCACTC CTTCGCCTGA GCCGCCTCCA 3660
AGTGTCTGCC GGTTCGCCGT GCCTCCTGGT CACACGAACA ATGGGTCTGA TTCCTCCGAC 3720
TCGGAGTACA GCTCTCAGAC CACGGTGTCT GGCATCAGTG AGGAGCTCAG GCAATACGAA 3780
GCACAGCAGG GTGCCGGAGG CCCTGCCAC CAAGTGATTG TGGAAGCCAC AGAAAACCT 3840
GTCITTTGCC GGTCCACTGT GGTCCATCCG GACTCCAGAC ATCAGCCTCC CTTGACCCCT 3900

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CGGCAACAGC CCCACCTGGA CTCTGGCTCC TTGTCCCCTG GACGGCAAGG CCAGCAGCCT 3960
CGAAGGGATC CCCCTAGAGA AGGCTTGC GG CCACCCCCCT ACAGACCGCG CAGAGACGCT 4020
TTTGAAATTT CTACTGAAGG GCATTCTGGC CCTAGCAATA GGGACCGCTC AGGGCCCCGT 4080
GGGGCCCCGT CTCACAACCC TCGGAACCCA ACGTCCACCG CCATGGGCAG CTCTGTGCCC 4140
AGCTACTGCC AGCCCATCAC CACTGTGACG GCTTCTGCTT CGGTGACTGT TGCTGTGCAT 4200
CCCCCGCCTG GACCTGGGCG CAACCCCCGA GGGGGGCCCT GTCCAGGCTA TGAGAGCTAC 4260
CCTGAGACTG ATCACGGGGT ATTTGAGGAT CCTCATGTGC CTTTTCATGT CAGGTGTGAG 4320
AGGAGGGACT CAAAGGTGGA GGTATAGAG CTACAGGACG TGAATGTGA GGAGAGGCCG 4380
TGGGGGAGCA GCTCCAAC TG AGGTAATTA AATCTGAAG CAAAGAGGCC AAAGATTGGA 4440
AAGCCCCGCC CCCACCTCTT TCCAGAATG CTGGAAGAGA ACTGCTTGA ATTATGGAA 4500
GGCAGTTCAT TGTTACTGTA ACTGATTGTA TTATTKGTG AAATATTCT ATAAATATT 4560
AARAGGTGTA CACATGTAAT ATACATGGAA ATGCTGTACA GTCTATTTC TGGGGCCTCT 4620
CCACTCCTGC CCCAGAGTGG GGAGACCACA GGGGCCCTTT CCCCTGTGTA CATTGGTCTC 4680
TGTGCCACAA CCAAGCTTAA CTTAGTTTAA AAAAAATCT CCCAGCATAT GTCGCTGCTG 4740
CTTAAATATT GTATAATTTA CTTGTATAAT TCTATGCAA TATTGCTTAT GTAATAGGAT 4800
TATTGTGAAA GGTTTCTGT TAAATATTT TAAATTTGCA TATCACAACC CTGTGGTAGG 4860
ATGAATTGTT ACTGTAACT TTTGAACAG CTATGCGTGG TAATTGTTA ACGAGCAGAC 4920
ATGAAGAAAA CAGGTTAATC CCAGTGGCTT CTCTAGGGT AGTTGTATAT GGTTGCGATG 4980
GGTGGATGTG TGTGTGCATG TGACTTTCCA ATGTACTGTA TTGTGGTTTG TTGTGTGTGT 5040
TGCTGTTGTT GTTCAATTTG GTGTTTTGG TTGCTTGTG TGATCTTAGC TCTGGCCTAG 5100
GTGGGCTGGG AAGGTCCAGG TCTTTTCTG TCGTGATGCT GGTGGAAAGG TGACCCCAAT 5160
CATCTGTCCT ATTCTCTGGG ACTATTC 5187

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1434 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein,

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Ala Ser Ala Gly Asn Ala Ala Gly Ala Leu Gly Arg Gln Ala Gly
1 5 10 15
Gly Gly Arg Arg Arg Arg Thr Gly Gly Pro His Arg Ala Ala Pro Asp

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20	25	30
Arg Asp Tyr Leu His Arg Pro Ser Tyr Cys Asp Ala Ala Phe Ala Leu		
35	40	45
Glu Gln Ile Ser Lys Gly Lys Ala Thr Gly Arg Lys Ala Pro Leu Trp		
50	55	60
Leu Arg Ala Lys Phe Gln Arg Leu Leu Phe Lys Leu Gly Cys Tyr Ile		
65	70	75
80		
Gln Lys Asn Cys Gly Lys Phe Leu Val Val Gly Leu Leu Ile Phe Gly		
85	90	95
Ala Phe Ala Val Gly Leu Lys Ala Ala Asn Leu Glu Thr Asn Val Glu		
100	105	110
Glu Leu Trp Val Glu Val Gly Gly Arg Val Ser Arg Glu Leu Asn Tyr		
115	120	125
Thr Arg Gln Lys Ile Gly Glu Glu Ala Met Phe Asn Pro Gln Leu Met		
130	135	140
Ile Gln Thr Pro Lys Glu Glu Gly Ala Asn Val Leu Thr Thr Glu Ala		
145	150	155
160		
Leu Leu Gln His Leu Asp Ser Ala Leu Gln Ala Ser Arg Val His Val		
165	170	175
Tyr Met Tyr Asn Arg Gln Trp Lys Leu Glu His Leu Cys Tyr Lys Ser		
180	185	190
Gly Glu Leu Ile Thr Glu Thr Gly Tyr Met Asp Gln Ile Ile Glu Tyr		
195	200	205
Leu Tyr Pro Cys Leu Ile Ile Thr Pro Leu Asp Cys Phe Trp Glu Gly		
210	215	220
Ala Lys Leu Gln Ser Gly Thr Ala Tyr Leu Leu Gly Lys Pro Pro Leu		
225	230	235
240		
Arg Trp Thr Asn Phe Asp Pro Leu Glu Phe Leu Glu Glu Leu Lys Lys		
245	250	255
Ile Asn Tyr Gln Val Asp Ser Trp Glu Glu Met Leu Asn Lys Ala Glu		
260	265	270
Val Gly His Gly Tyr Met Asp Arg Pro Cys Leu Asn Pro Ala Asp Pro		
275	280	285
Asp Cys Pro Ala Thr Ala Pro Asn Lys Asn Ser Thr Lys Pro Leu Asp		
290	295	300
Val Ala Leu Val Leu Asn Gly Gly Cys Gln Gly Leu Ser Arg Lys Tyr		
305	310	315
320		
Met His Trp Gln Glu Glu Leu Ile Val Gly Gly Thr Val Lys Asn Ala		
325	330	335
Thr Gly Lys Leu Val Ser Ala His Ala Leu Gln Thr Met Phe Gln Leu		
340	345	350
Met Thr Pro Lys Gln Met Tyr Glu His Phe Arg Gly Tyr Asp Tyr Val		
355	360	365

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Ser His Ile Asn Trp Asn Glu Asp Arg Ala Ala Ala Ile Leu Glu Ala
 370 375 380
 Trp Gln Arg Thr Tyr Val Glu Val Val His Gln Ser Val Ala Pro Asn
 385 390 395 400
 Ser Thr Gln Lys Val Leu Pro Phe Thr Thr Thr Thr Leu Asp Asp Ile
 405 410 415
 Leu Lys Ser Phe Ser Asp Val Ser Val Ile Arg Val Ala Ser Gly Tyr
 420 425 430
 Leu Leu Met Leu Ala Tyr Ala Cys Leu Thr Met Leu Arg Trp Asp Cys
 435 440 445
 Ser Lys Ser Gln Gly Ala Val Gly Leu Ala Gly Val Leu Leu Val Ala
 450 455 460
 Leu Ser Val Ala Ala Gly Leu Gly Leu Cys Ser Leu Ile Gly Ile Ser
 465 470 475 480
 Phe Asn Ala Ala Thr Thr Gln Val Leu Pro Phe Leu Ala Leu Gly Val
 485 490 495
 Gly Val Asp Asp Val Phe Leu Leu Ala His Ala Phe Ser Glu Thr Gly
 500 505 510
 Gln Asn Lys Arg Ile Pro Phe Glu Asp Arg Thr Gly Glu Cys Leu Lys
 515 520 525
 Arg Thr Gly Ala Ser Val Ala Leu Thr Ser Ile Ser Asn Val Thr Ala
 530 535 540
 Phe Phe Met Ala Ala Leu Ile Pro Ile Pro Ala Leu Arg Ala Phe Ser
 545 550 555 560
 Leu Gln Ala Ala Val Val Val Val Phe Asn Phe Ala Met Val Leu Leu
 565 570 575
 Ile Phe Pro Ala Ile Leu Ser Met Asp Leu Tyr Arg Arg Glu Asp Arg
 580 585 590
 Arg Leu Asp Ile Phe Cys Cys Phe Thr Ser Pro Cys Val Ser Arg Val
 595 600 605
 Ile Gln Val Glu Pro Gln Ala Tyr Thr Glu Pro His Ser Asn Thr Arg
 610 615 620
 Tyr Ser Pro Pro Pro Pro Tyr Thr Ser His Ser Phe Ala His Glu Thr
 625 630 635 640
 His Ile Thr Met Gln Ser Thr Val Gln Leu Arg Thr Glu Tyr Asp Pro
 645 650 655
 His Thr His Val Tyr Tyr Thr Thr Ala Glu Pro Arg Ser Glu Ile Ser
 660 665 670
 Val Gln Pro Val Thr Val Thr Gln Asp Asn Leu Ser Cys Gln Ser Pro
 675 680 685
 Glu Ser Thr Ser Ser Thr Arg Asp Leu Leu Ser Gln Phe Ser Asp Ser
 690 695 700

Ser Leu His Cys Leu Glu Pro Pro Cys Thr Lys Trp Thr Leu Ser Ser
 705 710 715 720
 Phe Ala Glu Lys His Tyr Ala Pro Phe Leu Leu Lys Pro Lys Ala Lys
 725 730 735
 Val Val Val Ile Leu Leu Phe Leu Gly Leu Leu Gly Val Ser Leu Tyr
 740 745 750
 Gly Thr Thr Arg Val Arg Asp Gly Leu Asp Leu Thr Asp Ile Val Pro
 755 760 765
 Arg Glu Thr Arg Glu Tyr Asp Phe Ile Ala Ala Gln Phe Lys Tyr Phe
 770 775 780
 Ser Phe Tyr Asn Met Tyr Ile Val Thr Gln Lys Ala Asp Tyr Pro Asn
 785 790 795 800
 Ile Gln His Leu Leu Tyr Asp Leu His Lys Ser Phe Ser Asn Val Lys
 805 810 815
 Tyr Val Met Leu Glu Glu Asn Lys Gln Leu Pro Gln Met Trp Leu His
 820 825 830
 Tyr Phe Arg Asp Trp Leu Gln Gly Leu Gln Asp Ala Phe Asp Ser Asp
 835 840 845
 Trp Glu Thr Gly Arg Ile Met Pro Asn Asn Tyr Lys Asn Gly Ser Asp
 850 855 860
 Asp Gly Val Leu Ala Tyr Lys Leu Leu Val Gln Thr Gly Ser Arg Asp
 865 870 875 880
 Lys Pro Ile Asp Ile Ser Gln Leu Thr Lys Gln Arg Leu Val Asp Ala
 885 890 895
 Asp Gly Ile Ile Asn Pro Ser Ala Phe Tyr Ile Tyr Leu Thr Ala Trp
 900 905 910
 Val Ser Asn Asp Pro Val Ala Tyr Ala Ala Ser Gln Ala Asn Ile Arg
 915 920 925
 Pro His Arg Pro Glu Trp Val His Asp Lys Ala Asp Tyr Met Pro Glu
 930 935 940
 Thr Arg Leu Arg Ile Pro Ala Ala Glu Pro Ile Glu Tyr Ala Gln Phe
 945 950 955 960
 Pro Phe Tyr Leu Asn Gly Leu Arg Asp Thr Ser Asp Phe Val Glu Ala
 965 970 975
 Ile Glu Lys Val Arg Val Ile Cys Asn Asn Tyr Thr Ser Leu Gly Leu
 980 985 990
 Ser Ser Tyr Pro Asn Gly Tyr Pro Phe Leu Phe Trp Glu Gln Tyr Ile
 995 1000 1005
 Ser Leu Arg His Trp Leu Leu Leu Ser Ile Ser Val Val Leu Ala Cys
 1010 1015 1020
 Thr Phe Leu Val Cys Ala Val Phe Leu Leu Asn Pro Trp Thr Ala Gly
 1025 1030 1035 1040

70

Ile Ile Val Met Val Leu Ala Leu Met Thr Val Glu Leu Phe Gly Met
 1045 1050 1055
 Met Gly Leu Ile Gly Ile Lys Leu Ser Ala Val Pro Val Val Ile Leu
 1060 1065 1070
 Ile Ala Ser Val Gly Ile Gly Val Glu Phe Thr Val His Val Ala Leu
 1075 1080 1085
 Ala Phe Leu Thr Ala Ile Gly Asp Lys Asn His Arg Ala Met Leu Ala
 1090 1095 1100
 Leu Glu His Met Phe Ala Pro Val Leu Asp Gly Ala Val Ser Thr Leu
 1105 1110 1115 1120
 Leu Gly Val Leu Met Leu Ala Gly Ser Glu Phe Asp Phe Ile Val Arg
 1125 1130 1135
 Tyr Phe Phe Ala Val Leu Ala Ile Leu Thr Val Leu Gly Val Leu Asn
 1140 1145 1150
 Gly Leu Val Leu Leu Pro Val Leu Leu Ser Phe Phe Gly Pro Cys Pro
 1155 1160 1165
 Glu Val Ser Pro Ala Asn Gly Leu Asn Arg Leu Pro Thr Pro Ser Pro
 1170 1175 1180
 Glu Pro Pro Pro Ser Val Val Arg Phe Ala Val Pro Pro Gly His Thr
 1185 1190 1195 1200
 Asn Asn Gly Ser Asp Ser Ser Asp Ser Glu Tyr Ser Ser Gln Thr Thr
 1205 1210 1215
 Val Ser Gly Ile Ser Glu Glu Leu Arg Gln Tyr Glu Ala Gln Gln Gly
 1220 1225 1230
 Ala Gly Gly Pro Ala His Gln Val Ile Val Glu Ala Thr Glu Asn Pro
 1235 1240 1245
 Val Phe Ala Arg Ser Thr Val Val His Pro Asp Ser Arg His Gln Pro
 1250 1255 1260
 Pro Leu Thr Pro Arg Gln Gln Pro His Leu Asp Ser Gly Ser Leu Ser
 1265 1270 1275 1280
 Pro Gly Arg Gln Gly Gln Gln Pro Arg Arg Asp Pro Pro Arg Glu Gly
 1285 1290 1295
 Leu Arg Pro Pro Pro Tyr Arg Pro Arg Arg Asp Ala Phe Glu Ile Ser
 1300 1305 1310
 Thr Glu Gly His Ser Gly Pro Ser Asn Arg Asp Arg Ser Gly Pro Arg
 1315 1320 1325
 Gly Ala Arg Ser His Asn Pro Arg Asn Pro Thr Ser Thr Ala Met Gly
 1330 1335 1340
 Ser Ser Val Pro Ser Tyr Cys Gln Pro Ile Thr Thr Val Thr Ala Ser
 1345 1350 1355 1360
 Ala Ser Val Thr Val Ala Val His Pro Pro Pro Gly Pro Gly Arg Asn
 1365 1370 1375

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Pro Arg Gly Gly Pro Cys Pro Gly Tyr Glu Ser Tyr Pro Glu Thr Asp
 1380 1385 1390

His Gly Val Phe Glu Asp Pro His Val Pro Phe His Val Arg Cys Glu
 1395 1400 1405

Arg Arg Asp Ser Lys Val Glu Val Ile Glu Leu Gln Asp Val Glu Cys
 1410 1415 1420

Glu Glu Arg Pro Trp Gly Ser Ser Ser Asn
 1425 1430

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 11 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Ile Ile Thr Pro Leu Asp Cys Phe Trp Glu Gly
 1 5 10

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Leu Ile Val Gly Gly
 1 5

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Pro Phe Phe Trp Glu Gln Tyr

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 28 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GGACGAATTC AARGINCAYC ARYTNTGG

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(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 26 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GGACGAATTC CYTCCCARAA RCANTC

26

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 27 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GGACGAATTC YTNGANTGYT TYTGGA

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(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 31 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CATACCAGCC AAGCTTGTCTN GGCCARTGCA T

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(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5288 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GAATTCGGG GACCGCAAGG AGTGCCGCGG AAGCGCCCGA AGGACAGGCT CGCTCGGCGC	60
GCCGGCTCTC GCTCTTCCGC GAACTGGATG TGGGCAGCGG CGGCCGAGA GACCTCGGGA	120
CCCCCGCGCA ATGTGGCAAT GGAAGGCGCA GGGTCTGACT CCCCGGCAGC GGCCGCGGCC	180
GCAGCGSCAG CAGCGCCCGC CGTGTGAGCA GCAGCAGCGG CTGGTCTGTC AACCAGAGCC	240
CGAGCCCGAG CAGCCTGCGG CCAGCAGCGT CCTCGCAAGC CGAGCGCCCA GGCGCGCCAG	300
GAGCCCGCAG CAGCGGCAGC AGCGCGCCGG GCGGCCCGGG AAGCCTCCGT CCCCGCGGCG	360
GCGGCGGCGG CGGCGGCGGC AACATGGCCT CGGCTGGTAA CGCCGCCGAG CCCAGGACC	420
GCGGCGGCGG CGGCGGCGGC TGTATCGGTG CCCCGGAGC GCCGGCTGGA GGCGGGAGGC	480
GCAGACGGAC GGGGGGGCTG CGCCGTGCTG CCGCGCCGGA CCGGGACTAT CTGCACCGGC	540
CCAGCTACTG CGACGCCGCC TTCGCTCTGG AGCAGATTTC CAAGGGGAAG GCTACTGGCC	600
GGAAAGCGCC ACTGTGGCTG AGAGCGAAGT TTCAGAGACT CTTATTAA CTGGTTGTT	660
AAATCAAAA AAATGCGGC AAGTTCCTGG TTGTGGGCTT CCTCATATTT GGGGCTTCG	720
CGGTGGGATT AAAAGCAGCG AACCTCGAGA CCAACGTGGA GGAGCTGTGG GTGGAAGTTG	780
GAGGACGAGT AAGTCGTGAA TTAAATTATA CTCGCCAGAA GATTGGAGAA GAGGCTATGT	840
TTAATCCTCA ACTCATGATA CAGACCCCTA AAGAAGAAGG TGCTAATGTC CTGACCACAG	900
AAGCGCTCCT ACAACACCTG GACTCGGCAC TCCAGGCCAG CCGTGTCCAT GTATACATGT	960
ACAACAGGCA GTGGAAATTG GAACATTTGT GTTACAAATC AGGAGAGCTT ATCACAGAAA	1020
CAGCTTACAT GGATCAGATA ATAGAATATC TTTACCCTTG TTTGATTATT ACACCTTTGG	1080
ACTGCTTCTG GGAAGGGGCG AAATTACAGT CTGGGACAGC ATACCTCCTA GGTAACCTC	1140

CTTTGCGGTG GACAAACTTC GACCCTTTGG AATTCCTGGA AGAGTTAAAG AAAATAAACT	1200
ATCAAGTGGA CAGCTGGGAG GAAATGCTGA ATAAGGCTGA GGTGGTTCAT GGTACATGG	1260
ACCGCCCTG CCTCAATCCG GCCGATCCAG ACTGCCCCGC CACAGCCCCC AACAAAAAT	1320
CAACCAAACC TCTTGATATG GCCCTTGTTT TGAATGGTGG ATGTCATGGC TTATCCAGAA	1380
AGTATATGCA CTGGCAGGAG GAGTTGATTG TGGGTGGCAC AGTCAAGAAC AGCACTGGAA	1440
AACCTGTCAG CGCCCATGCC CTGCAGACCA TGTTCAGTT AATGACTCCC AAGCAAATGT	1500
ACGAGCACTT CAAGGGGTAC GAGTATGTCT CACACATCAA CTGGAACGAG GACAAAGCGG	1560
CAGCCATCCT GGAGGCCTGG CAGAGGACAT ATGTGGAGGT GGTTCATCAG AGTGTGCGAC	1620
AGAACTCCAC TCAAAAGGTG CTTTCCTTCA CCACCACGAC CCTGGACGAC ATCCTGAAAT	1680
CCTTCTCTGA CGTCAGTGTG ATCCGCGTGG CCAGCGGCTA CTTACTCATG CTCGCTATG	1740
CCTGTCTAAC CATGCTGCGC TGGGACTGCT CCAAGTCCCA GGGTSCCGTG GGGCTGGCTG	1800
GGTTCCTGCT GGTTCGACTG TCAGTGGCTG CAGGACTGGG CCTGTCTCTA TTGATCGGAA	1860
TTTCTTTTAA CGCTGCAACA ACTCAGGTTT TGCCATTCTT CGCTCCTGGT GTTGGTGTGG	1920
ATGATGTTTT TCTTCTGGCC CACGCCTTCA GTGAAACAGG ACAGAATAAA AGAATCCCTT	1980
TTGAGGACAG GACCGGGGAG TGCCCTGAAGC GCACAGGAGC CAGCGTGGCC CTCACGTCCA	2040
TCAGCAATGT CACAGCCTTC TTCATGGCCG CGTTAATCCC AATTCCCGCT CTGCGGGCGT	2100
TCTCCCTCCA GGCAGCGGTA GTAGTGGTGT TCAATTTTGC CATGGTTCTG CTCATTTTTC	2160
CTGCAATTCT CAGCATGGAT TTATATCGAC GCGAGGACAG GAGACTGGAT ATTTTCTGCT	2220
GTTTTACAAG CCCCTGCGTC AGCAGAGTGA TTCAGGTTGA ACCTCAGGCC TACACCGACA	2280
CACACGACAA TACCCGCTAC AGCCCCCAC CTCCCTACAG CAGCCACAGC TTGCCCCATG	2340
AAACGCAGAT TACCATGCAG TCCACTGTCC AGCTCCGCAC GGAGTACGAC CCCCACACGC	2400
ACGTGTACTA CACCACCGCT GAGCCGCGCT CCGAGATCTC TGTGCAGCCC GTCACCGTGA	2460
CACAGGACAC CCTCAGCTGC CAGAGCCCAG AGAGCACCAG CTCCACAAGG GACCTGCTCT	2520
CCCAGTTCTC CGACTCCAGC CTCCACTGCC TCGAGCCCCC CTGTACGAAG TGGACACTCT	2580
CATCTTTTGC TGAGAAGCAC TATGCTCCTT TCCTCTTGAA ACCAAAAGCC AAGGTAGTGG	2640
TGATCTTCCT TTTTCTGGGC TTGCTGGGGG TCAGCCTTTA TGGCACCACC CGAGTGAGAG	2700
ACGGGCTGGA CCTTACGGAC ATTGTACCTC GGGAAACCAG AGAATATGAC TTTATTGCTG	2760
CACAATTCAA ATACTTTTCT TTCTACAACA TGTATATAGT CACCCAGAAA GCAGACTACC	2820
CGAATATCCA GCACTTACTT TACGACCTAC ACAGGAGTTT CAGTAACGTG AAGTATGTCA	2880
TGTTGGAAGA AAACAAACAG CTTCCCAAAA TGTGGCTGCA CTAATTCAGA GACTGGCTTC	2940
ATTTACTTCA GGATGCATTT GACAGTGAAT GGGAAACCGG GAAAATCATG CCAACAATTT	3000
ACAAGAATGG ATCAGACGAT GGAGTCCTTG CCTACAAACT CCTGGTGCAA ACCGGCAGCC	3060

GCSATAAGCC CATCGACATC AGCCAGTTGA CTAACAGCG TCTGGTGGAT GCAGATGGCA	3120
TCATTAATCC CAGCGCTTTC TACATCTACC TGACGGCTTG GGTCAGCAAC GACCCCGTCG	3180
CGTATGCTGC CTCCCAGGCC AACATCCGGC CACACCGACC AGAATGGGTC CACGACAAAG	3240
CCGACTACAT GCCTGAAACA AGGCTGAGAA TCCCGGCAGC AGAGCCCATC GAGTATGCCC	3300
AGTTCCCTTT CTACCTCAAC GGGTTGCGGG ACACCTCAGA CTTTGTGGAG GCAATTGAAA	3360
AAGTAAGGAC CATCTGCAGC AACTATACGA GCCTGGGGCT GTCCAGTTAC CCCAACGGCT	3420
ACCCCTTCCT CTTCTGGGAG CAGTACATCG GCCTCCGCCA CTGGCTGCTG CTGTTTCATCA	3480
GCGTGGTGTT GGCCTGCACA TTCCTCGTGT GCGCTGTCTT CCTTCTGAAC CCCTGGACGG	3540
CCGGGATCAT TGTGATGGTC CTGGCGCTGA TGACGGTCGA GCTGTTCCGC ATGATGGGCC	3600
TCATCGGAAT CAAGCTCAGT GCCGTGCCCC TGGTCATCCT GATCGCTTCT GTTGGCATAG	3660
GAGTGGAGTT CACCGTTCAC GTTGTCTTGG CCTTCTGAC GGCCATCGGC GACAAGAACC	3720
GCAGGGCTGT GCTTGCCCTG GAGCACATGT TTGCACCCGT CCTGGATGGC GCCGTGTCCA	3780
CTCTGCTGGG AGTGCTGATG CTGGCGGGAT CTGAGTTCGA CTTATTGTC AGGTATTCT	3840
TTGCTGTGCT GGCATCCTC ACCATCCTCG GCGTCTCAA TGGGCTGGTT TTGCTTCCCG	3900
TGCTTTTGTC TTTCTTTGGA CCATATCCTG AGGTGTCTCC AGCCAACGGC TTGAACCGCC	3960
TGCCCCACCC CTCCCTGAG CCACCCCCCA GCGTGGTCCG CTTGCGCATG CCGCCCGGCC	4020
ACACGCACAG CGGGTCTGAT TCCTCCGACT CGGAGTATAG TTCCAGACG ACAGTGTACG	4080
GCCTCAGCGA GGAGCTTCGG CACTACGAGG CCCAGCAGGG CGCGGGAGGC CCTGCCCCACC	4140
AAGTGATCGT GGAAGCCACA GAAAACCCCG TCTTCGCCCCA CTCCACTGTG GTCCATCCCC	4200
AATCCAGGCA TCACCCACCC TCGAACCCGA GACAGCAGCC CCACCTGGAC TCAGGGTCCC	4260
TGCTTCCCGG ACGGCAAGGC CAGCAGCCCC GCAGGGACCC CCCCAGAGAA GGCTTGTGGC	4320
CACCCCTCTA CAGACCGCGC AGAGACGCTT TTGAAATTTT TACTGAAGGG CATTCTGGCC	4380
CTAGCAATAG GGCCCGCTGG GGCCCTCGCG GGGCCCGTTC TCACAACCCT CGGAACCCAG	4440
CGTCCACTGC CATGGGCAGC TCCGTGCCCC GCTACTGCCA GCCCATCACC ACTGTGACGG	4500
CTTCTGCCTC CGTGAATGTC GCCGTGCACC CGCCGCTGT CCCTGGGCCT GGGCGGAACC	4560
CCCGAGGGGG ACTCTGCCCC GGCTACCCTG AGACTGACCA CGGCCTGTTT GAGGACCCCC	4620
ACGTGCCTTT CCACGTCCGG TGTGAGAGGA GGGATTGCAA GGTGGAAGTC ATTGAGCTGC	4680
AGGACGTGGA ATGCGAGGAG AGGCCCCGGG GAAGCAGCTC CAACTGAGGG TGATTAAAT	4740
CTGAAGCAA GAGGCCAAAG ATTGGAACCC CCCACCCCC ACCTCTTTCC AGAACTGCTT	4800
GAAGAGAACT GGTGTGAGTT ATGGAAAAGA TGCCCTGTGC CAGGACAGCA GTTCATTGTT	4860
ACTGTAACCG ATTGTATTAT TTTGTAAAT ATTTCTATAA ATATTTAAGA GATGTACACA	4920

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TGTGTAATAT AGGAAGGAAG GATGTAAAGT GGTATGATCT GGGGCTTCTC CACTCCTGCC	4980
CCAGAGTGTG GAGGCCACAG TGGGGCCTCT CCGTATTTGT GCATTGGGCT CCGTGCCACA	5040
ACCAAGCTTC ATTAGTCTTA AATTTCAGCA TATGTTGCTG CTGCTTAAAT ATTGTATAAT	5100
TTACTTGTAT AATTCTATGC AAATATTGCT TATGTAATAG GATTATTTTG TAAAGGTTTC	5160
TGTTTAAAT ATTTTAAATT TGCATATCAC AACCTGTGG TAGTATGAAA TGTTACTGTT	5220
AACCTTCAAA CACGCTATGC GTGATAATTT TTTTGTTTAA TGAGCAGATA TGAAGAAAGC	5280
CCGGAATT	5288

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1447 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Met	Ala	Ser	Ala	Gly	Asn	Ala	Ala	Glu	Pro	Gln	Asp	Arg	Gly	Gly	Gly	1	5	10	15
Gly	Ser	Gly	Cys	Ile	Gly	Ala	Pro	Gly	Arg	Pro	Ala	Gly	Gly	Gly	Arg	20	25	30	
Arg	Arg	Arg	Thr	Gly	Gly	Leu	Arg	Arg	Ala	Ala	Ala	Pro	Asp	Arg	Asp	35	40	45	
Tyr	Leu	His	Arg	Pro	Ser	Tyr	Cys	Asp	Ala	Ala	Phe	Ala	Leu	Glu	Gln	50	55	60	
Ile	Ser	Lys	Gly	Lys	Ala	Thr	Gly	Arg	Lys	Ala	Pro	Leu	Trp	Leu	Arg	65	70	75	80
Ala	Lys	Phe	Gln	Arg	Leu	Leu	Phe	Lys	Leu	Gly	Cys	Tyr	Ile	Gln	Lys	85	90	95	
Asn	Cys	Gly	Lys	Phe	Leu	Val	Val	Gly	Leu	Leu	Ile	Phe	Gly	Ala	Phe	100	105	110	
Ala	Val	Gly	Leu	Lys	Ala	Ala	Asn	Leu	Glu	Thr	Asn	Val	Glu	Glu	Leu	115	120	125	
Trp	Val	Glu	Val	Gly	Gly	Arg	Val	Ser	Arg	Glu	Leu	Asn	Tyr	Thr	Arg	130	135	140	
Gln	Lys	Ile	Gly	Glu	Glu	Ala	Met	Phe	Asn	Pro	Gln	Leu	Met	Ile	Gln	145	150	155	160
Thr	Pro	Lys	Glu	Glu	Gly	Ala	Asn	Val	Leu	Thr	Thr	Glu	Ala	Leu	Leu	165	170	175	
Gln	His	Leu	Asp	Ser	Ala	Leu	Gln	Ala	Ser	Arg	Val	His	Val	Tyr	Met				

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515	520	525
Lys Arg Ile Pro Phe Glu Asp Arg Thr Gly Glu Cys Leu Lys Arg Thr 530 535 540		
Gly Ala Ser Val Ala Leu Thr Ser Ile Ser Asn Val Thr Ala Phe Phe 545 550 555 560		
Met Ala Ala Leu Ile Pro Ile Pro Ala Leu Arg Ala Phe Ser Leu Gln 565 570 575		
Ala Ala Val Val Val Val Phe Asn Phe Ala Met Val Leu Leu Ile Phe 580 585 590		
Pro Ala Ile Leu Ser Met Asp Leu Tyr Arg Arg Glu Asp Arg Arg Leu 595 600 605		
Asp Ile Phe Cys Cys Phe Thr Ser Pro Cys Val Ser Arg Val Ile Gln 610 615 620		
Val Glu Pro Gln Ala Tyr Thr Asp Thr His Asp Asn Thr Arg Tyr Ser 625 630 635 640		
Pro Pro Pro Pro Tyr Ser Ser His Ser Phe Ala His Glu Thr Gln Ile 645 650 655		
Thr Met Gln Ser Thr Val Gln Leu Arg Thr Glu Tyr Asp Pro His Thr 660 665 670		
His Val Tyr Tyr Thr Thr Ala Glu Pro Arg Ser Glu Ile Ser Val Gln 675 680 685		
Pro Val Thr Val Thr Gln Asp Thr Leu Ser Cys Gln Ser Pro Glu Ser 690 695 700		
Thr Ser Ser Thr Arg Asp Leu Leu Ser Gln Phe Ser Asp Ser Ser Leu 705 710 715 720		
His Cys Leu Glu Pro Pro Cys Thr Lys Trp Thr Leu Ser Ser Phe Ala 725 730 735		
Glu Lys His Tyr Ala Pro Phe Leu Leu Lys Pro Lys Ala Lys Val Val 740 745 750		
Val Ile Phe Leu Phe Leu Gly Leu Leu Gly Val Ser Leu Tyr Gly Thr 755 760 765		
Thr Arg Val Arg Asp Gly Leu Asp Leu Thr Asp Ile Val Pro Arg Glu 770 775 780		
Thr Arg Glu Tyr Asp Phe Ile Ala Ala Gln Phe Lys Tyr Phe Ser Phe 785 790 795 800		
Tyr Asn Met Tyr Ile Val Thr Gln Lys Ala Asp Tyr Pro Asn Ile Gln 805 810 815		
His Leu Leu Tyr Asp Leu His Arg Ser Phe Ser Asn Val Lys Tyr Val 820 825 830		
Met Leu Glu Glu Asn Lys Gln Leu Pro Lys Met Trp Leu His Tyr Phe 835 840 845		
Arg Asp Trp Leu Gln Gly Leu Gln Asp Ala Phe Asp Ser Asp Trp Glu 850 855 860		

Thr Gly Lys Ile Met Pro Asn Asn Tyr Lys Asn Gly Ser Asp Asp Gly
 865 870 875 880
 Val Leu Ala Tyr Lys Leu Leu Val Gln Thr Gly Ser Arg Asp Lys Pro
 885 890 895
 Ile Asp Ile Ser Gln Leu Thr Lys Gln Arg Leu Val Asp Ala Asp Gly
 900 905 910
 Ile Ile Asn Pro Ser Ala Phe Tyr Ile Tyr Leu Thr Ala Trp Val Ser
 915 920 925
 Asn Asp Pro Val Ala Tyr Ala Ala Ser Gln Ala Asn Ile Arg Pro His
 930 935 940
 Arg Pro Glu Trp Val His Asp Lys Ala Asp Tyr Met Pro Glu Thr Arg
 945 950 955 960
 Leu Arg Ile Pro Ala Ala Glu Pro Ile Glu Tyr Ala Gln Phe Pro Phe
 965 970 975
 Tyr Leu Asn Gly Leu Arg Asp Thr Ser Asp Phe Val Glu Ala Ile Glu
 980 985 990
 Lys Val Arg Thr Ile Cys Ser Asn Tyr Thr Ser Leu Gly Leu Ser Ser
 995 1000 1005
 Tyr Pro Asn Gly Tyr Pro Phe Leu Phe Trp Glu Gln Tyr Ile Gly Leu
 1010 1015 1020
 Arg His Trp Leu Leu Leu Phe Ile Ser Val Val Leu Ala Cys Thr Phe
 1025 1030 1035 1040
 Leu Val Cys Ala Val Phe Leu Leu Asn Pro Trp Thr Ala Gly Ile Ile
 1045 1050 1055
 Val Met Val Leu Ala Leu Met Thr Val Glu Leu Phe Gly Met Met Gly
 1060 1065 1070
 Leu Ile Gly Ile Lys Leu Ser Ala Val Pro Val Val Ile Leu Ile Ala
 1075 1080 1085
 Ser Val Gly Ile Gly Val Glu Phe Thr Val His Val Ala Leu Ala Phe
 1090 1095 1100
 Leu Thr Ala Ile Gly Asp Lys Asn Arg Arg Ala Val Leu Ala Leu Glu
 1105 1110 1115 1120
 His Met Phe Ala Pro Val Leu Asp Gly Ala Val Ser Thr Leu Leu Gly
 1125 1130 1135
 Val Leu Met Leu Ala Gly Ser Glu Phe Asp Phe Ile Val Arg Tyr Phe
 1140 1145 1150
 Phe Ala Val Leu Ala Ile Leu Thr Ile Leu Gly Val Leu Asn Gly Leu
 1155 1160 1165
 Val Leu Leu Pro Val Leu Leu Ser Phe Phe Gly Pro Tyr Pro Glu Val
 1170 1175 1180
 Ser Pro Ala Asn Gly Leu Asn Arg Leu Pro Thr Pro Ser Pro Glu Pro
 1185 1190 1195 1200

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Pro Pro Ser Val Val Arg Phe Ala Met Pro Pro Gly His Thr His Ser
 1205 1210 1215
 Gly Ser Asp Ser Ser Asp Ser Glu Tyr Ser Ser Gln Thr Thr Val Ser
 1220 1225 1230
 Gly Leu Ser Glu Glu Leu Arg His Tyr Glu Ala Gln Gln Gly Ala Gly
 1235 1240 1245
 Gly Pro Ala His Gln Val Ile Val Glu Ala Thr Glu Asn Pro Val Phe
 1250 1255 1260
 Ala His Ser Thr Val Val His Pro Glu Ser Arg His His Pro Pro Ser
 1265 1270 1275 1280
 Asn Pro Arg Gln Gln Pro His Leu Asp Ser Gly Ser Leu Pro Pro Gly
 1285 1290 1295
 Arg Gln Gly Gln Gln Pro Arg Arg Asp Pro Pro Arg Glu Gly Leu Trp
 1300 1305 1310
 Pro Pro Leu Tyr Arg Pro Arg Arg Asp Ala Phe Glu Ile Ser Thr Glu
 1315 1320 1325
 Gly His Ser Gly Pro Ser Asn Arg Ala Arg Trp Gly Pro Arg Gly Ala
 1330 1335 1340
 Arg Ser His Asn Pro Arg Asn Pro Ala Ser Thr Ala Met Gly Ser Ser
 1345 1350 1355 1360
 Val Pro Gly Tyr Cys Gln Pro Ile Thr Thr Val Thr Ala Ser Ala Ser
 1365 1370 1375
 Val Thr Val Ala Val His Pro Pro Pro Val Pro Gly Pro Gly Arg Asn
 1380 1385 1390
 Pro Arg Gly Gly Leu Cys Pro Gly Tyr Pro Glu Thr Asp His Gly Leu
 1395 1400 1405
 Phe Glu Asp Pro His Val Pro Phe His Val Arg Cys Glu Arg Arg Asp
 1410 1415 1420
 Ser Lys Val Glu Val Ile Glu Leu Gln Asp Val Glu Cys Glu Glu Arg
 1425 1430 1435 1440
 Pro Arg Gly Ser Ser Ser Asn
 1445

5 WHAT IS CLAIMED IS:

1. An isolated nucleic acid encoding a *patched* protein other than *Drosophila melanogaster patched* protein, or fragment of at least about 12 nt in length thereof, as other than an intact chromosome.
- 10 2. An isolated nucleic acid according to Claim 1 wherein said *patched* protein is mosquito, butterfly or beetle.
3. An isolated nucleic acid according to Claim 1, wherein said *patched* protein is a mammalian protein.
4. An isolated nucleic acid according to Claim 3, wherein said *patched* protein is human.
- 15 5. In isolated nucleic acid according to Claim 3, wherein said *patched* protein is mouse.
6. An expression cassette comprising a transcriptional initiation region functional in an expression host, a nucleic acid having a sequence of o the isolated nucleic acid according to Claim 1 under the transcriptional regulation of said transcriptional initiation region, and a transcriptional termination region functional in said expression host.
- 20 7. A cell comprising an expression cassette according to Claim 6 as part of an extrachromosomal element or integrated into the genome of a host cell as a result of introduction of said expression cassette into said host cell and the cellular progeny of said host cell.
8. A method for producing *patched* protein, said method comprising growing a cell
- 25 according to Claim 7, whereby said *patched* protein is expressed; and isolating said *patched* protein free of other proteins.
9. A purified polypeptide composition comprising at least 50 weight % of the protein present as a *patched* protein or a fragment thereof, other than *Drosophila melanogaster patched* protein.
- 30 10. A purified polypeptide composition according to Claim 9, wherein said *patched* protein is a mammalian protein.
11. A purified polypeptide composition according to Claim 10, wherein said *patched* protein is human.
12. A purified polypeptide composition according to Claim 10, wherein said *patched* protein
- 35 is mouse.
13. A monoclonal antibody binding specifically to a *patched* protein other than *Drosophila melanogaster patched* protein.
14. A method for diagnosing a genetic predisposition for at least one of developmental abnormalities and cancer in an individual, the method comprising:
 - 40 — detecting the presence of a predisposing mutation in a *patched* gene in the germline of said individual,
 - wherein the presence of said predisposing mutation indicates that said individual has a genetic predisposition for at least one of developmental abnormalities and

5 cancer.

15. A method according to Claim 14, wherein said genetic predisposition is basal cell nevus syndrome.
- 10 16. A method according to Claim 14, wherein said detecting step comprises analyzing the DNA of said individual.
17. A method according to Claim 14, wherein said detecting step comprises functional analysis of patched protein function.
18. A method according to Claim 14, wherein said detecting step comprises detecting antibody binding to abnormal patched protein.
- 15 19. A method for characterizing the phenotype of a tumor, the method comprising:
 - detecting the presence of an oncogenic patched mutation in said tumor, wherein the presence of said oncogenic mutation indicates that said tumor has a patched-associated phenotype.
20. A method according to Claim 19, wherein said tumor is a carcinoma.
- 20 21. A method according to Claim 20, wherein said carcinoma is a basal cell carcinoma.
22. A method according to Claim 19, wherein said detecting step comprises analyzing the DNA of said tumor.
23. A method according to Claim 19, wherein said detecting step comprises functional analysis of patched protein function.
- 25 24. A method according to Claim 19, wherein said detecting step comprises detecting antibody binding to abnormal patched protein.
25. A genetically engineered mammalian cell predisposed to develop basal cell carcinoma as a result of transfection of said mammalian cell with at least one DNA construct comprising an altered patched or *hedgehog* gene.

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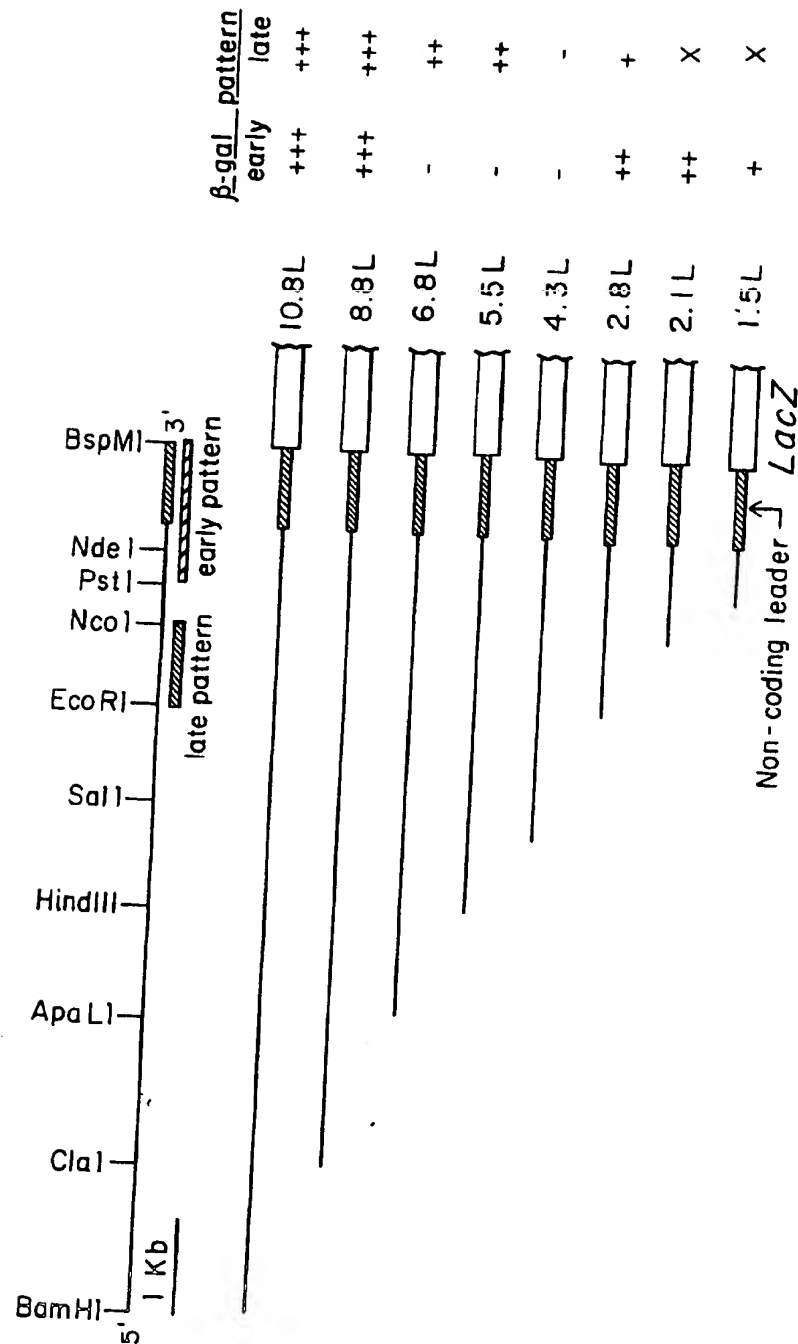
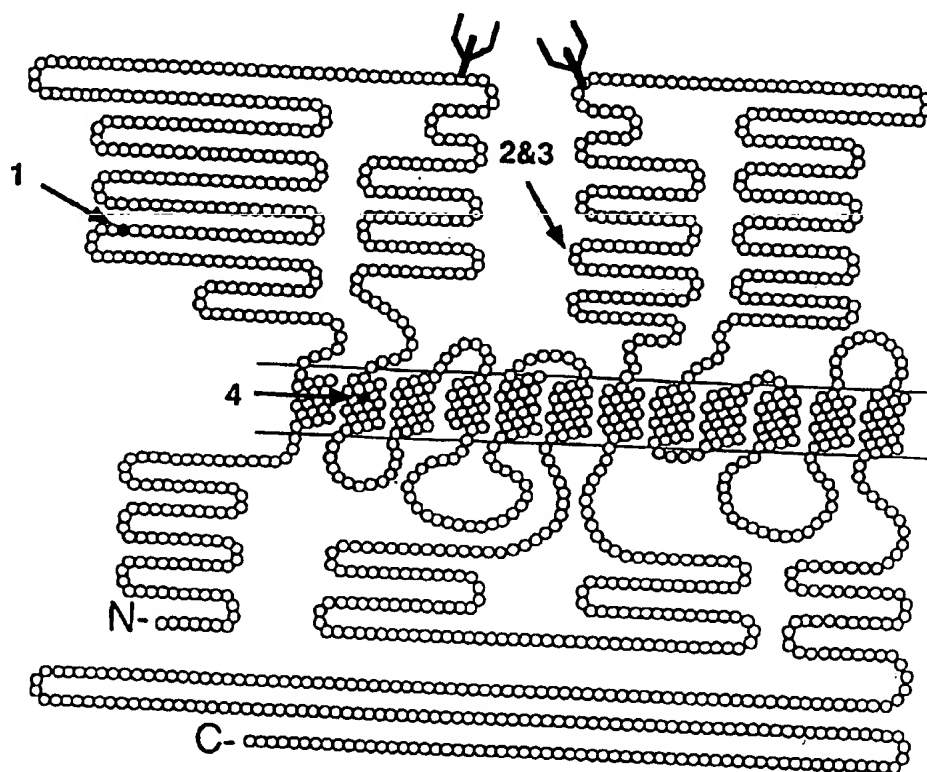


FIG. 1

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*FIG. 2*

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